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The goal of the project was to develop a battery of tests of social behavior and performance for nonhuman primates that would be sensitive to the effects of CW-related chemicals considered for use as antidotes or prophylactics against CW agents. Different procedures for assessing social behavior are described and evaluated, as are a number of tests for emotional reactivity, complex problem solving, and operant performance. Data are presented on changes in plasma hormone levels in response to manipulations of social and performance variables designed to induce stress in the subjects. The suitability of the test battery for use in studying CW-related chemicals was evaluated using an antidote (atropine sulphate), a therapeutic (diazepam), and control drugs (caffeine and atropine methyl nitrate). A set of tests to be included in the battery, along with some alternatives, is presented.

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FOREWORD

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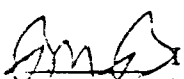
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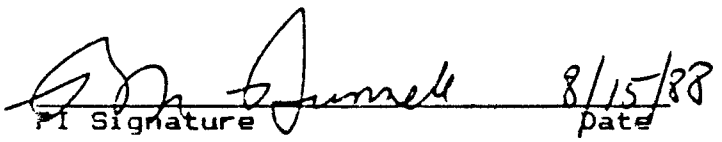
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Introduction

This report summarizes work accomplished during a three year project, the goal of which was to develop a battery of tests of social behavior and performance for nonhuman primates that would be sensitive to the effects of CW-related chemicals considered for use as antidotes or therapeutics against CW agents. Different procedures for assessing social behavior are described and evaluated, as are a number of tests for emotional reactivity, complex problem solving, and operant performance. Data are presented on changes in plasma hormone levels in response to manipulations of social and performance variables designed to induce stress in the subjects. The suitability of the test battery for use in studying CW-related chemicals was evaluated using an antidote (atropine sulphate), a therapeutic (diazepam), and control drugs (caffeine and atropine methyl nitrate). A set of tests to be included in the battery, along with some alternatives, is presented along with a proposed schedule for administering the battery.

The specific objectives of the project were: (1) To develop and evaluate a set of behavioral tests for studying social behavior, individual performance, and the relationships between individual performance and social behavior in nonhuman primates. (2) To evaluate the utility of this battery of tests by examining the effects of some CW-related chemicals that might be used as antidotes or therapeutics for CW agents on social behavior and performance. (3) To develop procedures and provide facilities for testing the long term behavioral sequalae of non-lethal exposure of nonhuman primates to CW agents.

The majority of the behavioral testing was done with 28 adult and subadult male cynomolgous macaque monkeys (*Macaca fascicularis*) who comprised the adult male social hierarchies of four captive groups of animals. Two of the groups were breeding troops containing females, juveniles and infants in addition to the adult males. The other two groups were all-male troops. Several procedures for gathering and analyzing social behavior data were evaluated in terms of their utility for drug studies as were different methods of manipulating the amount and kind of social behavior exhibited by the monkeys.

In addition to examining agonistic behaviors in considerable detail, particular attention was paid to affiliative social behavior in order to evaluate naturally occurring cooperative behavior in the groups as a possible dependent variable for inclusion in the test battery. Cooperative behavior was also studied in conjunction with group performance on an operant schedule in the laboratory. Other laboratory tasks evaluated included several appetitive operant schedules as well as a response suppression paradigm utilizing footshock, tests of responsiveness to novel environmental

stimuli, and a complex problem solving task which involved both object quality learning sets and reversal learning. A locomotor activity test was devised for studying drug effects on activity and this was used to determine the initial dosages to be used with each drug employed during the evaluation of the behavioral tests. Throughout the project, a series of assays of plasma hormones was used to monitor the animals' responses to physical and social stress.

In selecting drugs to be used as antidotes and therapeutics against chemical warfare (CW) agents, there is a natural concern for the potential effects of these drugs on behavior and performance. While such effects may be considered secondary to the primary objectives of minimizing or preventing the life threatening consequences of exposure to CW agents, it is important that information be available about the ways in which either acute or chronic doses of antidotes and prophylactics might affect performance on assigned tasks. This is especially true because such drugs may be taken, whether by design or accident, in the absence of exposure to agent. In addition, since many military tasks require social interaction, coordination, and cooperation, it is useful to identify those side effects which might interfere with social behavior and group oganization. Since most species of nonhuman primates are highly social, and since these animals are capable of performing at a high level on wide variety of behavioral tasks, they provide an animal model of considerable utility for tests of drug effects. It should be noted that a given drug may have different effects on benavior depending upon the social organization of a group and the social status of the monkeys receiving the drug (e.g., Delgado, Grau, Delgado-Garcia and Rodero, 1976). The identification and analysis of such drug/behavior interactions can be very important. Finally, an advantage of a nonhuman primate model over the use of human subjects in tests of this nature lies with the virtually continuous monitoring and control that is possible over all aspects of the animal's experiences. The groups of monkeys used in the present project had been involved in noninvasive behavioral studies for almost nine years prior to the fall of 1983 and extensive social and performance data were available as background.

In choosing the behavioral tests to be evaluated a number of factors were considered. First, the emphasis was placed upon the performance of well learned tasks rather than the acquisition of new skills. Second, the tasks were selected to tap a variety of different response dimensions in order to maximize the possibility of detecting drug effects on performance. Third, the tasks chosen were sufficiently demanding that, for the most part, they allowed the animals to exhibit either performance enhancement or performance decrement with respect to each subject's own baseline data. Fourth, there were certain motivational considerations: Although most of the tasks involved using food as a positive reinforcer,

tests involving avoidance and punishment were also evaluated, and tests of emotionality and general activity devised.

Thus, the operant tasks evaluated involved one reinforcement schedule which required witholding responses and relatively low response rates and others which produced moderate and high rates of steady responding, as well as specific response patterns and timing. A multiple schedule designed to produce behavioral contrast effects was employed and failures of reinforcement were utilized to examine the animals' response to frustration. Moderate levels of footshock administered during performance on appetitive schedules were used to evaluate response suppression to an aversive stimulus. In another kind of task, the animals were required to enter and explore an open field arena with and without the presence of novel objects in the field. Their latency to enter the field, their responses to the novel objects, and their locomotor activity in the field were recorded. This task was designed as a test of emotional and motivational factors. Yet another test of locomotor activity was developed and used for testing drug effects on locomotor activity and coordination. A complex problem solving task was used which examined performance on object quality learning sets and reversal learning. It was designed to provide data on both habit formation and strategy utilization. This task also included a false-reversal procedure which attempted to tap the animals' responses to unexpected failure of their response strategies. In the complex problem solving task, raisins, a preferred food, were used as reinforcers and food deprivation was not required to motivate the performance of the monkeys.

The social behavior of the monkeys belonging to the two all-male troops and the two breeding troops was monitored throughout the project. Particular attention was given to agonistic behaviors, i.e., aggressive and submissive behaviors, and to affiliative behaviors, e.g., approaches, solicitation of grooming, allogrooming, enlisting, etc., which promote group cohesion and frequently require cooperation between individuals. Analyses of the direction of submissive behaviors were used to reveal the social organizaton of the male dominance hierarchies and, in the breeding troops, the structure and social ranking of the female matrilines. Methods for manipulating both social status and the amount and kind of social behavior through animal removal and replacement procedures were evaluated. While the two breeding troops and one of the all-male troops were housed as groups throughout the project, members of the other all-male troop were normally housed in individual cages and put together in various combinations of individuals for tests of dyadic and triadic interactions. This troop was also used to study social responses to strange monkeys, to test drug effects on individuals when the same individuals were both the dominant and subordinate members of social pairs, and to examine the operant performance of the animals in the social group

situation. Animals from this group were also used in our efforts to develop a laboratory test of cooperative behavior.

Since some of our earlier work with these animals had demonstrated a relationship between a monkey's social rank and its performance on various laboratory tasks, the performance measures and the social behavior measures obtained during this project were examined for such correlations. We were particularly interested in seeing if the effects of a drug on a monkey's performance differed as a function of the animal's social status in its group. The appearance of such interactions would be of considerable importance in selecting tests for inclusion in the battery.

Finally, because social situations involving high levels of agonistic activity as well as certain of the laboratory performance tasks were presumed to be stressful, the use of plasma hormone measures as indicants of stress was evaluated for this species. Most of the work was done on cortisol, which is an indicant of mild stress and general arousal, and prolactin, which responds in a graded fashion to moderate and intense stressors. We evaluated circadian effects on baseline hormone levels and examined the effects of repeated sampling on hematocrit readings in developing protocols for use with studies of the effects of social stress.

On page 6 of the original Request for Quotations (RFQ DAMD17-83-Q-0007, dated 3 February 1983) it was stated that the project was to be an exploratory project concerned with behavioral test development and not a project for drug screening, per se. The data collected on the project reflect this emphasis on the selection and evaluation of behavioral test procedures. In the studies involving the administration of drugs, the primary objective was to determine the sensitivity of the tests to drug administration, the utility of the procedures for repetitive studies as might be required in chronic drug administration or in evaluating a series of drugs over a brief period of time, and the efficiency with which each tests might be incorporated into a battery of tests to be used in studies of drug development. In consequence, the evaluation of some tests and the effects of drugs on those tests have been quite thorough. In other cases, tests have been eliminatd from consideration because they proved impractical for inclusion in the battery or because they seemed to add little to the information provided by other behavioral measures. Typically, the studies of drug effects on such measures are incomplete or absent. Finally, there were some tests which were deemed promising candidates for inclusion in the battery but for which parametric drug testing was not conducted because the animals concerned were needed for use in evaluating additional behavioral measures. During the winter of 1986, the COTR for the project, COL C. F. Tyner, paid a site visit to the project and informed us that there was little liklihood that the project would be continued beyond the initial three year period

because the Command was abandoning work with behavioral animal models for CW problems. He recommended that we concentrate our research on studies of diazepam for the remainder of the contract and abandon plans to study some of the additional drugs suggested in the contract. We agreed to do this, made reference to this in our quarterly report for the first quarter of 1986, and, at the request of the acquisitions office, sent a letter to this effect via the COTR indicating our acceptance of this modification in the research program.

A list of all of the social and performance tasks evaluated during the project is given in Table 1.

Table 1

Social and Performance Tasks Evaluated

SOCIAL BEHAVIOR:

Outdoor Groups:	1. Baseline - Scan and Focal Procedures;	3 Troops
	2. Removal and Replacement Procedures;	3 Troops
	3. Stress Hormones	
Laboratory: (Feeder on/off)	1. Baseline - Scan and Focal Procedures;	1 Troop
	2. Dyads; Group Pairs (15 Pairs)	
	3. Triads; Group Pairs + strangers	
	4. Group; Group + Strangers	
	5. Stress Hormones	

PERFORMANCE:

Open Field:	1. Empty Open Field	
	2. Open Field + Novel Objects	
	3. Stress Hormones	
Problem Solving: (WGTA)	1. Object Quality Reversal Learning Sets	
	2. False Reversal Procedure	
	3. Social Preference Testing	
Operant:		
Appetitive:	1. DRL	
	2. Fixed Interval	
	3. Random Interval	
	4. Random Interval with Omission of Reinforcement + Stress Hormones	
	5. Multiple Random Interval	
	6. Fixed Ratio + Social	
	7. Operant Cooperative	
Aversive:	8. Response Suppression + Stress Hormones	
	9. Free Operant Avoidance	

Materials, Methods and Data

A. Monkey Colony:

The subjects used in the project consisted of approximately 90 *Macaca fascicularis* monkeys. These animals had been used in an earlier project involving the identification of relationships between social behavior and performance on laboratory tasks (Bunnell, 1982). The monkeys were divided into four groups. Two of these, called "T-Troop" and "NT-Troop", were breeding groups containing all age and sex classes of animals. The other two groups were small, all-male troops. "I-Troop" consisted of eight adult males that had been together for several years prior to the beginning of the project. "C-Troop", which contained six young adult and subadult males drawn from NT-Troop, was formed after the project began. T-, NT- and I-Troops were housed together in large outdoor compounds. The C-Troop males were housed in individual cages in the laboratory and were put together in various combinations of animals at different times. The composition of the various groups at the end of the first year of the project (30 September, 1984) is given in Table 2:

Table 2

Group Composition as of 30 September 1984
(Number of monkeys in each age/sex category.)*

	Adult		Subadult		Juvenile		Infant	
TROOP:	M	F	M	F	M	F	M	F
"T" N = 46:	6	16	3	5	1	3	7	5
"NT" N = 31:	7	12	3	0	2	1	3	3
"I" N = 8:	8	0						
"C" N = 6:	4	0	2	0				
TOTAL N = 91:	25	28	8	5	3	4	10	8

* Males (M) over 6 years old and females (F) over 4 years old are classified as adults. Males 4-6 and females 4 years old are subadults. Juveniles are over 1 year old (both sexes)(Angst, 1975).

The outdoor compounds used to house T-, NT-, and I-Troops were 14.1 m long, 3.1 m wide, and 2.0 m high. Each compound was equipped with perches, swings, and a water fountain and contained an observer station, 1.6 m square, in the center from which observations of social behavior were recorded. The compounds were connected to heated and airconditioned indoor quarters by runways 1.2 m in cross section. The runways were partially covered to provide shelter from rain and sun when the animals were outside. The indoor quarters consisted of cages 6.1 m long x 1.2 m wide x 2.2 m high which were equipped with water fountains and perches. Small guillotine doors on the sides of these cages were used to collect the animals in transport boxes for testing in the laboratory. Guillotine doors between the indoor cages and the runways, and between the runways and the compounds, allowed the animals to be moved to different sections of the living quarters during social testing and daily cleaning.

The 6 males of C-Troop were housed in a battery of individual cages in a separate colony room in the laboratory. An adjacent suite contained a cage, measuring 1.8 m x 1.8 m x 1.8 m, in one room and an observer station, equipped with one way windows, in the other. The C-Troop monkeys were brought from their colony cages and placed in this cage for studies of activity and social behavior.

Yet another room contained 18 individual cages that were used as a holding facility during laboratory testing.

The behavioral testing performed in the laboratory required that the monkeys serving as subjects be removed from their social groups each day, weighed, and brought to the test apparatus. They were also adapted to a restraint device used to hold the animals while blood was drawn for assay for stress hormones. The capture and restraint procedure was made a part of the daily routine for all animals undergoing experimental testing. A series of blood draws for assays of plasma cortisol and plasma prolactin was used to monitor the individual monkey's adaptation to the capture and handling process.

B. Activity Tests and Drug Dose Selection:

Procedures for observing general activity and for selecting the initial doses of the drugs to be used in the project were developed and standardized using the C-Troop males. The animals were released individually into the C-Troop observation cage (described earlier) and observed through the one-way glass windows. In recording locomotor movement, the cage was divided into 8 equal cubes, 0.9 m per side, and the observer recorded the number of moves from cube to cube during a test. The different behaviors exhibited by the animals were also recorded using a rating scale similar to the social

behavior inventory that is described in the next section - the animals often interacted with their images in the one-way glass. The rating scale also contained additional codes for various behaviors that were directed toward the environment. After 10 min of activity data were obtained, the observer donned a rubber fright mask and entered the room with the monkey. Activity and behavior in response to the masked observer were recorded for 90 sec. The test was concluded by having the observer present the monkey with a threatening stimulus - either a live snake or a length of garden hose. In establishing the initial doses of drugs to be employed in the social and performance tests, the monkeys were observed for an hour or more, beginning immediately following injection of the drug. In these observations, several fright mask presentations were made at intervals throughout the period. In addition to recording activity and behavior, the observer noted all physical changes as they appeared, such as changes in respiration, pupillary dilation, speed and coordination of movements, etc. The monkeys were then returned to their home cages and monitored by an observer until all overt signs of drug effects returned to normal. The animals were given food and water at this time and the latencies to eat and drink were recorded, as well as the kind of food that is eaten first (monkey biscuit, vegetable, fruit, etc.). In these tests, the onset of overt behavioral and physiological changes was used to determine the time to be used between administering a drug and the beginning of a behavioral test.

As soon as the observation cage was completed in early June of 1984, a series of activity tests were run with C-Troop males that had been given various doses of caffeine sodium benzoate intramuscularly (i.m.). Increases in locomotor exploratory behavior were noted at the 3-4 mg/kg range; depending on the animal involved, both increases and decreases appeared in the 10-16 mg/kg range, and there were no overt changes in the 0.6-1.0 mg/kg range. On the basis of these observations, our dose selection for the initial experiments was 0.8, 4, and 12 mg/kg of the salt. The smallest dose was used because it was supposed to be in the benzodiazepine antagonist range. Later in the summer, because of the individual differences we observed in the effects of caffeine on performance and because we were concerned about tolerance, additional doses, including 2, 8, 16, 24 and 36 mg/kg, were added to some of our protocols. Effects appeared with a short latency - on the order of 5-10 min - and 5 min was selected as the latency to be used between injection and the behavioral tests.

In the pilot work with atropine sulphate, i.m. doses of .08, .20, and .40 mg/kg produced maximum pupillary dilation and changes in respiration rate at about 15 min post injection. The two highest doses produced dose dependent decreases in

activity; however, the animals movements were well coordinated and they responded normally to the presence of the masked figure, giving lip smacks, some threats, and considerable flight and avoidance behavior. When the monkeys were returned to their home cages and fed, they showed an immediate interest in food and would eat fruit immediately, followed by pieces of sweet potato. Dry monkey biscuits were nibbled, but not consumed until two or more hours after return to the cage. Interestingly, though they ate moist food, they did not drink water immediately, nor was there any prolonged drinking at any time. It is as though they preferred the moist food because their mouths were dry, but there was no evidence of a centrally motivated thirst at these doses. Pupillary dilation typically lasted for several hours after return to the home cage, and this was the most persistent physical sign we observed. The first studies of the effects of atropine sulphate on performance utilized doses of .08, .20, and .40 mg/kg with a delay of 15 min between injection and testing. Subsequently, we added a dose of .032 mg/kg and dropped the .40 mg/kg dose. We also used delays of 30 and 60 min in some protocols.

Two pilot studies on the effects of diazepam on locomotor behavior and activity were conducted with the C-Troop males using doses ranging from 0.16 to 2.0 mg/kg (i.m.). At the highest doses, mild ataxia was seen and the animals had some problems climbing and leaping, suggesting a loss of visual-motor coordination. There were no observable effects on locomotion or activity with the lowest dose, but there was a dose dependent decrease in activity as the dose was increased above 0.5 mg/kg. there was a mild dipsogenic effect and the animals increased their food intake at intermediate doses, particularly when preferred food objects such as fruit and sweet potatoes were available. The doses selected for the initial experiments with diazepam were .16, .80, and 1.60 mg/kg. These covered a range which the literature suggested should be wide enough to detect subtle changes in social behavior as well as the more direct actions on locomotion, activity, feeding, and the like. In later experiments, particularly those involving chronic administraton of the drug, a dose of 0.4 mg/kg was used extensively. The delay between injection and the start of behavioral testing was held constant at 15 min in most of the experiments. The vehicle, which consisted of 40% propylene glycol and 10% ethanol as well as small amounts of buffer and preservative, was used as the placebo control in all of the diazepam experiments. The volume of injection never exceeded 1.60 ml.

C. Social Behavior:

Social Organization in *M. fascicularis*:

Sociality, defined as the tendency to associate with one's fellows and to form social groups, is characteristic of most primate species, including man. The ubiquity of primate societies makes the study of nonhuman primate groups of potential importance in understanding certain aspects of human social behavior and organization and offers the possibility of using this animal model as a tool for testing drug effects on social behavior. However, while most, if not all, nonhuman primate species evince only one of several possible social organizations, man is much more flexible in terms of the kinds of social organization exhibited in his societies. Thus, generalizations from studies of monkey social behavior must be made with caution; to gain the maximum benefit from such data, one must view the results in the context of the particular social organization exhibited by the species of primate being studied.

In *M. fascicularis*, there are two elements of the social organization of the monkey troops that are particularly important. The first of these is the social dominance hierarchy among the adult males, while the second consists of a social hierarchy of the matriarchies present in the group. In a matriarchy, an old female, her daughters, her daughters' daughters, etc. and their infant offspring form a social unit. Each matriarchical unit has a social rank within the hierarchy of matriarchies such that all members of a unit have the same social status as the matriarch and an increase or a drop in the social rank of the matriarch will be accompanied by a corresponding change in the status of the members of her matriarchy (Angst, 1975). The two kinds of hierarchies function to promote cohesion within the group. Once the dominance/subordination relationships are established, each animal knows his or her status with regard to very other animal in the group and overt aggression is greatly reduced. Maintenance of the social rank structure is accomplished by threats and submissive signals that do not involve physical contact and injuries are rare. In a stable social group, physical contacts generally involve mutual grooming, sitting with one another, hugging and embracing, sexual behavior, and other affiliative behaviors which promote group cohesion and appear to reduce tensions between individuals. The top-ranked, or "alpha" male plays a key role in controlling the activities of the other members of the social group (Wechsler, 1986).

Although one or more high ranking matriarchs may outrank some of the lower ranking adult males, the male dominance hierarchy and the hierarchy of matriarchies seem to function more or less independently within the group. Males tend to interact with other adult males in the hierarchy and with

females on an individual basis during grooming and copulation. (There may be some more subtle relationships between male status and the matriarchical structure, however. On occasion we have observed that the loss of a matriarch, in addition to resulting in the loss of status of her matriarchy, has been accompanied by a loss of rank of her adult sons in the male hierarchy (Bunnell, 1982).

Operationally, the social rank of an animal is defined in terms of defeats. The occurrence of a submissive behavior in a monkey indicates that the animal is inferior in rank to the animal toward which it directs the submissive signal. (Similarly, the animal is dominant over monkeys which direct submissive behaviors toward it.) The social rank hierarchy is constructed by noting the submissive member of all possible pairs of animals and combining this information to determine the relationships between each animal and all other members of the group. In captive groups of *M. fascicularis*, the hierarchy among adult males is usually linear, that is, all of the other males submit to the alpha male, the second-ranked ("beta") male sumits to the alpha male, but is in turn submitted to by the remainder of the group, and so on. Occasionally, an alliance between two males will occur and together these two animals will often hold a higher rank in the structure than they would as individuals. Reversals in rank can also be present such that animal E submits to D, F submits to E, but D submits to F. These departures from linearity are usually seen among the lower ranking animals of the group. With respect to the matriarchies, dominance submission relationships tend to be more complicated. As noted above, the female offspring of the matriarch usually hold the same rank that she does with respect to nonmembers of her matriarchy. Within the matriarchy, however, a dominance hierarchy exists among the adult females and their juvenile offspring appear to have the same ranks as their mothers in the subgroup.

The male dominance hierarchy was the main element of the social structure of the four monkey groups used in the experiments described in this report. However, the overall group structure was also determined and monitored for the two large breeding troops involved in the studies.

Social Data Collection:

Social behavior is scored using the behavior categories given in Table 3. The observers record the code for the animal exhibiting the behavior, a code for the behavior itself, and then a code for the animal that is the recipient of the behavior. The two procedures utilized in gathering data are the "group scan" and the "focal animal" techniques. In a group scan, the observer watches the entire group and records every behavior that occurs as it happens; a modified version of a group scan involves looking at each monkey in sequence and recording what it is doing at the instant it is scanned. The

focal animal procedure involves attending to only one animal for a period of time and recording the direction and nature of all behavior it either does or receives during that time.

Table 3

M. fascicularis Behavior Categories

Agonistic Behaviors:

Aggressive
- Chase
- Threat (open-mouth)
- Charge
- Slap
- Bite

Submissive
- Avoid
- Grimace
- Squeal
- Flee

Other Agonistic
- Lid
- Lip Smack
- Enlist
- Demonstrate

Sexual Behaviors:

- Sexual Present
- Mount (no thrusting)
- Mount (with thrusting)
- Masturbate
- Genital Manipulation (other animal)
- Genital Sniff (other animal)

Affiliative Social Behaviors:

- Present to Groom
- Groom
- Ventral-Ventral Hug
- Ventral-Dorsal Hug
- Sit-Next-To (Physical contact)
- Play (not included in analysis)

Non-Social Behaviors:

- Self Groom
- Move
- Sit - No Social Interaction

In the analyses of the social data obtained by the scan techniques, a laboratory computer provided a listing of the frequencies of each behavior performed by each monkey and the frequencies with which it directed these behaviors to each of the other monkeys in the troop. These data were then used to produce a series of matrices describing the basic social organization and dynamics of the group. Usually, several days' data were combined in these analyses. In this procedure, the computer sorted all of the data and determined the social rank of each animal on the basis of who was defeated by whom, using the submissive behavior categories listed in Table 2. This defines the social dominance hierarchy for the troop. The computer then printed a series of six matrices in which the animals were listed in the order of their social rank. In each matrix, the frequency of occurrence of each behavior, or class of behaviors selected for inclusion in that matrix, was given for each animal with respect to every other animal in its troop. (We are limited to 24 x 24 matrices; in scoring the behavior in T- and NT-Troops, the behavior of the 23 oldest animals in each group was scored and the 24th slot was used to represent all the remaining infants and juveniles in the troop). Four of the six matrices were used to summarize the combinations of behaviors listed under the functional categories Aggressive, Submissive, Sexual, and Affiliative as given in Table 2. For the other two matrices, any individual behavior of interest could be selected. Thus, we might look at threat - a measure of noncontact aggression - in order to compare it with the matrix for overall aggression, or obtain separate matrices for grooming, which is included in the Other Social matrix and play, which is not. Examples of these matrices may be found in Appendix A.

The data from each focal animal observation were analyzed individually or summarized across observations to provide baseline information on response frequencies and directions to which the data from observations during experimental manipulations could be compared. In some instances, the matrix programs were used with the focal data by combining these data for several animals for one or more days. For other purposes, useful information was obtained by combining both scan and focal data in a single matrix analysis.

Experimental Manipulations of Social Behavior:

Social behavior within the groups was manipulated in several ways. The primary method utilized with the troops in the outdoor compounds was the removal and replacement of one or more adult males. Typically, the removal of a high ranking male results in an increase in the frequency of agonistic behavior within the group; this is sometimes accompanied by changes in the structure of the male dominance hierarchy. Once the social

behavior has restabilized, the absent animal can be reintroduced and behavior recorded during his subsequent reintegration into the group. Removal and replacement of low ranking animals, which produces little social upheaval, was used as a control in these manipulations. In the indoor tests of social behavior, various combinations of animals from C-Troop were used. These animals were housed individually, except when social testing was in progress. Social manipulations consisted of placing different combinations of 2 to 6 animals together at the same time and of introducing strangers, males and females, that were drawn from the other troops, to various combinations of C-Troop monkeys. For drug tests with C-Troop, particular emphasis was placed on the testing of the dyadic interactions of pairs of animals. With the six C-Troop monkeys, there were 15 possible combinations of pairs. Since each of the four monkeys which occupied the second through the fifth ranks in the dominance hierarchy was dominant over and subordinate to at least one other animal in the group, it was possible to test drug effects on both dominance and submission in the same animal by selecting the appropriate pairs for observation.

Social Data Analyses:

The use of the focal animal observation procedure is essential to the study of drug effects on social behavior since it ensures that each experimental subject is observed in the same way, and for the same length of time, during each session. The procedure does has disadvantages, however, in that social interactions between other members of the troop are not recorded as with the scan procedure. Information about such interactions is often critical for monitoring the social structure of the groups and the overall levels of different classes of social behaviors. Thus, it was necessary to incorporate both scan and focal procedures in gathering our social data. Prior to the beginning of the project, we had made only limited use of the focal animal procedure in our previous work with these groups. It was necessary to compare and contrast data obtained by the scan and focal animal techniques in order to determine the best combinations of the two procedures for achieving the objectives of the project. The questions asked involved:

1. The extent to which the social behavior matrices were equivalent when they were generated from data using focal animal as opposed to group scan techniques. Included in this question were subsidiary questions such as the number of focal observation periods in which only the adult males were observed that were required to define (a) the male dominance hierarchy in the troop and (b) the social ranks of the other animals in the troop that interacted with the focal males. A related question

involved the extent to which a change in the frequency of specific behaviors throughout the troop was accurately reflected by the frequencies of this behavior obtained from the focal male data; yet another was the identification of those behaviors that might not be recorded at all when the focal procedure was employed .

2. The relative sensitivity of both procedures for detecting short term changes in the social structure that might be induced by either removing or replacing animals in the troops or by administering a drug.

3. The frequency with which observations of either kind must be made in order to maintain an accurate picture of the social organization of the troop and provide a baseline against which the experimental manipulations could be imposed. Gathering these data is a very labor intensive operation and we were interested in determining the most efficient schedules for each experimental objective.

Several observers were trained to collect social data during the late winter and spring of the first contract year. For the rest of that year and during the first quarter of the second year, most of the data collected on each troop employed a single observer who used both scan and focal techniques during each observation period. The procedure used most frequently with the two large troops, T- and NT, began with a systematic scan in which the behavior of each of the 23 monkeys being scored was sampled in turn for 30 sec. This was followed by a 5-min focal observation of each adult male in the troop and then the observation period was concluded with another systematic 30-sec scan. The order in which the animals were observed was different each day for both types of observations. Thus, about 22 min of scan data and 30-40 min of focal data were obtained each day from T- and NT-Troops. In I-Troop, observations began with a general scan which lasted 10 min (20 min in a few instances) during which all social interactions between the animals were recorded as they occurred; this was followed by 3-min focals on each of the 8 monkeys and the session concluded with another 10-min general scan. As with the larger troops, the order in which the focal observations were made was changed each day.

Analyses of scan and focal data. For the 23 oldest monkeys whose social behavior was scored in T- and NT-Troops, there were 253 possible combinations of pairs excluding the "other" category animals which were all scored under one code. In order to have a complete picture of the social rank structure of these groups, the dominance/submission relationship between the members of each pair must be known. Several months of daily observations may be needed before all

possible dominance/submission relationships can be observed and noted. There are several reasons for this. Dominance/submission relationshionships, once established, tend to be relatively stable and require minimal overt agonistic behavior to maintain. There is a gradient in the expression of agonistic behavior such that the adult males and the highest ranking females and the members of their matriarchies show the greatest amount of these behaviors. Agonistic behavior within matriarchies and between lower ranking matriarchies is less frequent. Subadult and the older juvenile males tend to interact largely with each other and with young juveniles rather than with other troop members. Exceptions to this gradient can also occur, however, which sometimes makes the determination of the dominance/submission relationships between higher ranking animals difficult. In the adult male hierarchy, some animals may be virtual social isolates while others may have alliances that reduce the frequency of agonistic interactions between members of a particular pair so that weeks may go by before the observer can verify the relationship. Fortunately, changes in relationships are readily apparent because agonistic behavior increases during a change in rank and the increase may last for several days.

To help us evaluate the scan procedures used in gathering the social data during the summer and fall of the first year of the project, we used data obtained in some of our earlier work in which we had employed 40-min general scans in scoring the 24 oldest members of T-Troop (see Bur ell, 1982). These data were reanalyzed to determine the number of dominance/submission relationships that were actually observed across different numbers of observation periods and the kinds of relationships that were easily identified vs those which were rarely or never observed. Three months of data containing 15 general scan observations for June, 16 for July, and 14 for November, 1979 were examined. (Data for August-October of that year were not comparable and were not used since focal procedures were used in Augu t and group composition was manipulated in September and October.) The results are summarized in Table 4. A total of 538 submissive behaviors was recorded in June; this enabled us to resolve the dominance/submission relationships between 129 of the 276 pairs possible in the 24 x 24 matrix (47%). Adding the July data which contained 343 submissive responses increased the total number of resolved relationships to 170 (62%). Data from the third month, in which there were 480 submissive behaviors, increased the total number of identified relationships observed in three months to 204 (74%) for the 45 days of observations.

Identification of the dominance relationships between animals was most rapid among the higher ranking animals. In the first month, 90 submissive responses identified 93% (14/15) of the relationships between the six adult males present in the

troop. In the top 8 monkeys, which included to the two highest ranking females, 86% (24/28) of the dominance/submission relationships were actually observed. In the top half of the group -12 monkeys- 70% (46/66) of the relationships were observed. These figures confirm the gradient described above, since the number of relationships actually observed in the entire troop was just 47% during the first month. Subsequent observations during July and November primarily served to clarify the relationships among the lower ranking monkeys.

When we began the anaylses of the social data gathered in the first year of the present project, it became evident that cutting the scan observations by about 50% in order to include a series of focal observations in each observation period drastically reduced our ability to identify social rank relationships from the scan data. The next to last column of Table 4 contains the data for 31 days of observations of T-Troop obtained from late August through early November, 1984. (The total amount of observation time is approximately equal to that for one month - about 15 observations - during 1979). Only 204 submissive responses were recorded, enabling the identification of just 19% (48/253) of the dominance/submission relationships. Only 8/15 relationships among the 6 adult males could actually be confirmed from these data and the relatiohships among the higher ranking females and between these females and the adult males were not observed in many cases. In fact, it was not possible to specify the ordinal ranking of the top 8, let alone the top 12, animals in the group from these data alone. Adding the focal observation data to the scan data for this period improved the picture somewhat. The percentage of dominance/submission relationships increased to 28% (71/253) and 11/15 relationships among the adult males were observed. The improvement involved only the higher ranking animals - only 2 relationships were identified out a possible 66 among the bottom 12 monkeys in the group.

Since the daily 20-min scan observations obtained from the two large troops did not provide an efficient way of obtaining sufficient data to keep up with the social rank structure of the troops, the procedures were modified to provide more scan data. First, several weeks of data were gathered on T- and NT-Troops during April and May, 1985, using a systematic scan with each of the 23 monkeys being observed twice and with the observers instructed to record all agonistic activity whenever and wherever it occurred. Observation periods generally lasted between 45 and 55 min. Data from T-Troop for the month of May, 1985 are given in the last column of Table 4.

Table 4

Comparisons of Scan Data Obtained From T-Troop Using Different Scan Procedures
(1979 Data are Cumulative Across Three Months)

	40 Min Scans 1979			20 Min Scans 1984	45 Min Scans 1985
	June	+July	+Nov.	Aug.-Nov.	May
Number of Observations	15	31	45	31	20
# Submissive Behaviors	538	881 (+342)	1361 (+480)	204	720
Dominance/ Submission Relationships Identified	129/ 276	170/ 276 (+41)	204/ 276 (+34)	48/ 253	134/ 253
Intermale Relationships Identified	14/ 15	15/ 15 (+1)	15/ 15	8/ 15	13/ 15
Top Eight Relationships Identified	24/ 28	27/ 28 (+3)	27/ 28	Ranks Unknown	23/ 28
Top Twelve Relationships Identified	46/ 66	56/ 66 (+10)	62/ 66 (+6)	Ranks Unknown	48/ 66

A total of 720 submissive behaviors by the 23 monkeys being scored enabled us to actually identify 53% (134/253) of the possible dominance submission relationships from 21 days of observations. Although 3 of the 6 adult males in the group ranked in the bottom half of the hierarchy, 13 of the 15 intermale relationships were identified. (Actually, 11 of 15 were seen during the first 9 days of observations.) Once again, relationships were clearest among the higer ranking animals, with 23/28 relationships being verified among the top eight animals, 3 males and 5 females. Overall, the top 12 monkeys accounted for 116 of the 134 relationships identified. These data compare very favorably with that obtained from the 40 min general scans used with T-Troop in 1979. However, the May, 1985 scan data from NT-Troop produced only 100 submissive behaviors over 19 days of observations and these allowed the identificaton of only 11% (27/253) of the relationships, including 11 of the 21 dominance/submission relationships among

the 7 adult males. Adding 16 more days for June and July increased the totals to 22% (55/253) overall and to 17/21 of the intermale relationships. These figures are low, but are somewhat better than those obtained with the 20 min scans on NT-Troop in 1984. An 18 day sample from September, 1985 in which each observation period contained 40 min of systematic scans (in addition to 5-min focals) yielded only 93 submissive behaviors from the scan data but allowed us to verify 22 additional relationships which we had not seen before. Agonistic activity in this group was low from the spring of through the fall of 1985 and the social rank structure was quite stable for many months.

Although 40-50 min of scan data produced much more than twice as much information about the social rank structure than our 20 min scans, at least in T-Troop, extending the length of the daily observation periods much beyond 40 min did not produce proportionately more data about interanimal relationships. The agonistic interactions observed on any one day are likely to involve the same animals, so while extending the observation periods to 60 min or more increased the total frequency of aggressive and submissive behaviors recorded, we found that it did little to increase the identification of dominance/submission relationships.

The focal observation procedure is obviously not geared to producing complete dominance/submission matrices since the interactions between animals that are not themselves focal animals are excluded from consideration except as they happen to interact with the focals. Even among the animals that are being scored, the observer will miss interactions that may occur between monkeys that do not happen to be under observation at that moment in time. For example, when we examined 19 days of 10-min focal observations of 8 adult monkeys (6 males and 2 females) in T-Troop that were obtained in August, 1979, we found that, at the end of 19 days, submission had been recorded in only 12 of the possible 28 relationships among these 8 animals and in 23 more interactions between these 8 and the remainder of the troop. The 1979 scan data from any one of the other three months represented in Table 4 obviously does a better job of identifying dominance/submission relationships at varous levels of the group structure than the focal procedure. Similar findings were obtained from 19 days of 5-min focal data that were gathered on the same days that scan data were obtained in 1984. As noted earlier, combining scan and focal data from these days improved the percentage of dominance submission relationships that could be identified, at least among the higher ranking animals, but the combination did not provide a substitute for the information provided by additional scan data.

Since I-Troop contained only the 8 adult males, all of which could be scored as focal animals, we thought that the focal procedure might work better in identifying dominance/submission relationships than was the case in the two large troops. Twenty days of 5-min focal observation of each of the eight I-Troop males (40 min per day) during June, 1984 produced 84 submissive behaviors and allowed 20/28 relationships to be identified. By way of contrast, 18 days of 40-min general scan data obtained in June, 1985, yielded 166 submissive behaviors and identified 26/28 dominance/submission relationships. An analysis of the July, 1985 scan data showed that all 28 relationships appeared in the 21 days of data that contained 181 submissive responses. In fact, 27/28 relationships were identified from the first 11 days of scan data. Use of the scan procedure is clearly preferable for identifying dominance/submission relationships and reconstructing the social rank hierarchy. The July, 1985 data are reproduced in Appendix (A) which also serves to illustrate the social data matrices produced by the analyses we are using.

The comparisons of the 1984 T-Troop data with the 1979 data, the analyses and comparisons of the 1984 focal and scan data from all three troops, and the results of the extended scans obtained in the spring of 1985 led to a major change in the procedures used during the last 18 months of the project. Beginning in late May of 1985, daily observations of T- and NT-Troops were changed such that each observation period began and ended with a 20-min systematic scan. Four 5-min focal observations of the adult males in each troop were inserted in the middle of the observation period. Since there were 8 adult males in NT-Troop and 6 in T-Troop and only four focals were done each day, the order in which the animals were observed was rotated so that each male was a focal animal two or three times a week. To make up for the loss of focal animal data resulting from the increased scan time, subsequent experimental studies of drug effects on social behavior utilized two observers so that simultaneous scan and focal data were obtained throughout the observation period. This substantially improved the quality of the data obtained and compensated for the increased observer time required.

Manipulations of social rank. Earlier work (Bunnell, et al, 1979a, 1979b, 1980a, 1980b and Bunnell, 1982) had demonstrated relationships between social rank and performance on various laboratory tasks. On some tasks, high ranking animals outperformed low ranking animals while low ranking animals were better on certain other tasks. Performance tended to change as rank changed such that, when an animal gained or lost rank, performance shifted in the expected direction. Since drugs might affect performance differently as a function of rank, we were very interested in looking at procedures for inducing changes in the dominance hierarchies.

The primary procedure used in the experimental manipulation of social behavior involved the removal and replacement of males within a troop. Several deliberate removals and replacements were done with the two breeding troops and with the all male troop. In addition, occasions on which it was necessary to remove animals due to injury or illness were used as opportunites for studying removal and replacement effects. Also, there were several instances of spontaneous changes in the male dominance hierarchies of the three troops and these were used to evaluate the effects of rank changes on selected individuals in the groups.

Whether or not a given removal/replacement procedure produced a significant change in the male dominance hierarchy depended upon both the rank of the monkey being removed and the stability of the social organization of the group prior to the manipulation. A total of 15 removal/replacement manipulations of animals of varying rank (9 deliberate removals and another 6 resulting from injury or illness) were examined in detail. The length of time animals were out of their troops ranged from 8 days to 3 months (removals lasting less than 5 days previously had been shown to have minimal effects, Bunnell, 1982).

Only removal of the alpha male produced rearrangements in the structure of the male hierarchy and these changes were most noticeable in situations where there was instability in the troop (defined as high levels of agonistic activity) prior to the manipulation. Some examples: Late in the first year of the project, we removed the second (beta) and fifth ranked males in T-Troop, the third ranked male in NT-Troop and the alpha (first ranked) male in I-Troop for 8 days. Only the last manipulation produced a significant change in social organization as the third ranked male moved to the alpha position in I-troop. (Upon reintroduction, the former leader reassumed first rank.) Next, the alpha male (Barker) in NT-Troop was removed for 3 weeks. This produced both a sharp increase in agonistic behavior within the group and a disruption of the structure of the male hierarchy. Prior to the manipulation the NT-Troop hierarchy was less stable than those of the other two troops as the NT- males were exhibiting considerably more agonistic behavior than T- and I-Troop males. After Barker was removed the beta male, Eju, lost out to the third ranked male, Weed, who became the temporary alpha. Hobbit, the seventh ranked animal moved up two ranks. Eju, who had held the beta position because he had an alliance with Barker, fell into a tie with Allen in the rank below Weed. After several days, Weed was injured in an unobserved fight and had to be taken out of the troop for treatment of his wounds. Upon Barker's return, he was attacked by Allen. Allen and the fifth ranked animal, Tag, were injured and removed for treatment as Barker reestablished himself as the alpha male. The return of Barker also reestablished the original ranks among the bottom animals in the hierarchy. Weed

was returned two days after Barker and became the beta male, once again displacing Eju. Over the next three weeks, agonistic behavior remained at a high level and there were several more injuries which caused animals to be removed from the troop for one or more days before the situation stabilized.

In another series of manipulations involving I-Troop, which was relatively stable during this time, we removed Cracker, the fifth ranked male in I-Troop for 18 days, repeated this procedure with Gus, the alpha, and looked at the effect of the absence of Alabama, the beta male, during a 3 month removal. None of these manipulations produced any changes in the relative ranks of the monkeys within the hierarchy. Similarly, removal of Easy, the beta male in T-Troop, for treatment of an injury, resulted in no rank order changes during the 12 days he was out of the troop; this occurred during a period of low to moderate levels of agonistic behavior in this group. The results from the other removals and replacements were similar to those seen in these examples so far as the rank structure was concerned.

A more interesting and, for our purposes, more useful effect of the removal/replacement procedures involved changes in the frequencies and patterns of agonistic behaviors. In the manipulations of I-Troop described in the preceding paragraph, the removal and replacement of Cracker, a low ranking animal, had no effect on the frequency of agonistic interactions within the troop while he was out. During these 17 days we recorded a mean frequency of 12.3 agonistic behaviors in the group. However, on the day of his return, there were 28 such behaviors throughout the troop. The frequency of agonistic behavior returned to preintroduction levels the following day. Alabama, the beta male in I-Troop, was reintroduced after an absence of three months. there were 105 agonistic behaviors recorded on the day of his return compared with a mean of 10.1 for 6 days prior and 7.7 for 14 days after his return. Similarly, the reintroduction of Easy, the beta male of T-Troop after a 12 day absence produced 40 agonistic interactions, only 5 of these involved Easy, who reassumed his prior status without being challenged by the other males. This increase is in contrast to agonistic mean frequencies of 10.3 for the three days prior to and 10.7 for the three days after the reintroduction day.

During the seven days prior to the return of Gus, the alpha male of I-Troop, the mean daily frequency of agonistic behaviors was 11.3. The total of 53 submissive behaviors recorded during this time allowed us to verify 17/21 possible dominance/submission relationships. On the day of his reintroduction there were 55 agonistic behaviors and the 24 submissive responses identified 8/28 dominance/submission relationships, even though none of these involved Gus himself who made only one agonistic response, a threat (!). The mean

frequency of agonistic behaviors during the 14 days following Gus' return was 18.0 and the 197 submissive responses allowed us to confirm 26/28 dominance/submission relationships (see Table 5). With the troop intact during the next calendar month, there was a gradual decline toward preintroduction levels of agonistic behavior - 15 days of observations yielded a mean of 14.6 behaviors. During the last 15 months of the project, the general findings from the removal/reintroduction procedures were reconfirmed with I- and NT-Troops. Reintroduction produced increases in agonistic behavior throughout the troop and these encounters included many relationships other than those involving the reintroduced animal.

From these results we concluded that removal and replacement of selected males is an effective way of generating increases in agonistic behavior throughout the monkey groups. These effects were related to the status of the animal removed and replaced, the relative stability of the group at the time the manipulation was conducted, and the length of time the monkey was out of the troop. With a stable social structure, high ranking animals may be removed for two or more weeks and returned without seriously disturbing the rank hierarchy and with a low potential for physical injuries. In less stable groups, low ranking animals may also be used in this fashion, but the amount of agonistic behavior induced is low and the use of low ranking animals was not an effective way of generating agonistic behavior for use in studying drug effects on this class of social behaviors. On the other hand, the removal and replacement of high ranking males, particularly the alpha male, from an unstable group may produce profound changes in the structure of the group that can confound the interpretation of drug effects. In addition, the potential for injuries is increased, which is undesirable for both experimental and animal welfare considerations. The amount and intensity of agonistic behavior generated by the removal and replacement of the alpha male in NT-Troop, described above, interfered with both operant testing and drug testing in this group for several weeks, since stable baselines were unobtainable and several injured animals had to be removed from the testing program for varying periods of time.

Nonagonistic social behavior. The amount of affiliative behavior observed in a group might be expected to decrease as agonistic behavior increases following a removal and reintroduction manipulation. Although there is a decline in these behaviors, the change is small and most behaviors are present with sufficient frequency to allow the detection of both increases and decreases following drug or other experimental manipulations. Table 5 gives the mean I-Troop frequencies of affiliative, allogrooming, and sexual behaviors per monkey per day for the 7 days before Gus' reintroduction, the day of his reintroduction, and for the following 14 days

during the manipulation described earlier. There was a decrease in affiliative behaviors, including grooming, on the day of reintroduction and sexual behavior disappeared. During the following two weeks, the frequencies recovered to near the preintroduction levels. Mean sexual behaviors actually increased slightly. Intermale mounting in these animals is both a sign of affiliative behavior and a way in which interanimal relationships are confirmed.

Table 5

Social Behavior in I-Troop for 7 Days Before, During, and 14 Days After Gus' Reintroduction (1985). Data are Given as Mean Frequencies/Day for the Entire Troop

Behavior Category	Pre-Reintro (n=7) 7 days	Reintro (n=8) 1 day	Post-Reintro (n=8) 14 days
All Agonistic	11.3	55	18.0
Submissive	7.3	24	14.1
All Affiliative	50.8	55	62.4
Grooms*	23.8	20	23.2
Sexual	14.0	0	18.4
Dominance/ Submission	17/21	8/28	26/28

* Grooms are also included in the "Affiliative" category (See Table 2).

Social behavior in C-Troop. C-Troop, the group used in indoor testing of social behavior, initially consisted of five young males that were removed from NT-Troop in the spring of 1984. A sixth animal, also from NT-Troop, was added the following fall. The animals were housed in separate cages in a colony room assigned for that purpose. After completion of construction of the social testing cage, described in an earlier section of this report, the monkeys were individually adaptated to the test cage environment for several weeks before social testing was begun. During this period the procedures for the general activity test were developed, using the first five animals as subjects. Following the adaptation period, tests of general activity were conducted which were used for establishing the doses to be used in the caffeine and atropine studies. Social testing of the C-Troop monkeys was begun during the last two months of the first year of the project.

In the initial tests, the five monkeys were placed together in pairs for 10 min of behavioral observations. Each of the 10 possible pair combinations was observed each day and both social behavior and general activity were scored using the scan procedures described elsewhere in this report. The purpose of the initial tests of social behavior was to determine the dominance/subordination relationships among the 5 animals and to obtain frequency data for the social behavior categories so that drug effects on social behavior could then be observed in selected pairs of monkeys. However, two weeks of testing produced very little agonistic behavior among the animals despite the fact that they had been isolated from all physical contact with each other for over two months. We next put all five monkeys together as a group for 40 min a day and observed them using a combination of scan and focal observations for three weeks. During this time, efforts were made to increase the agonistic interactions by throwing fruit into the cage and by attaching a food box to the cage and observing the animals when they were food deprived. Using these procedures we were able to determine the dominance hierarchy although the frequency of agonistic behaviors remained low, even during competition for food or fruit. We then separated the animals for one week, retested their social behavior on three days during the following week, kept them apart for two more weeks, and resumed social testing. These brief separations did not produce significant increases in agonistic behavior as we had hoped they might.

One of the objectives of the project was to develop tests of cooperative behavior in the monkeys. One of the ways in which monkeys "cooperate" is to enlist the aid of other animals against their opponents during agonistic encounters. A detailed investigation of this "appeal-aggression" in captive M. fascicularis can be found in de Waal (1977). Enlisting is done using a stereotyped combination of gestures and facial expressions in which the animal that is doing the enlisting first threatens an opponent, turns his head and looks at the animal he is enlisting, and then directs another threat at the opponent. This may be repeated several time as the monkey advances toward the opponent, taking short steps and alternately threatening the opponent and glancing back at his ally until the ally joins the encounter. We tried to develop procedures which would produce enlisting behavior reliably so that we could use it as a dependent variable in studying cooperative behavior. We also wanted to see if we could increase the overall frequency of agonistic behaviors in these tests. The procedures involved placing both familiar and unfamiliar animals in with pairs of C-Troop males and recording the ensuing behaviors. In addition to tests with both dyads and triads, observations were made of the troop as a 6-animal social unit both alone and in the presence of unfamiliar males and females from other troops. Finally, an operant panel

containing a pellet feeder, manipulanda, cue lights, and a loudspeaker was placed on one wall of the social test cage and the social behavior of the animals, both in pairs and as a group, was observed during the delivery of food pellets on a 30 sec variable time (VT 30-sec) reinforcement schedule. Later, the monkeys were trained to press a lever to receive banana and their social behavior was observed while fixed ratio (FR) reinforcement schedules were in effect.

The first manipulation involved the brief introduction of a new animal to the five-member group. First, the animals were placed together in the observation cage daily for nine days. A five-min scan was followed by a five-min focal observation of each animal, in random order, and the session was concluded with another five min scan. On the 10th, a "strange" male, named Defeat, was introduced to the group following the completion of the regular observation period. Observations continued for 10 min with the new animal serving as the focal animal. (Defeat was the young adult, mentioned earlier, that had been removed from NT-Troop and kept in the laboratory for use as a stimulus animal. Since the C-Troop animals were originally taken from NT-Troop, Defeat was not a complete stranger, but the C-Troop males had had no contact with him for eight months prior to this test.) Two more days of observations followed, with the stranger absent. A series of blood samples were taken throughout this experiment and the results of the hormone assays will be presented in a later section of this report.

During the next five days of observations, the five C-Troop animals were put together in the social cage each morning and kept together for the rest of the day. Observations (5 min scan - 5 min individual focals - 5 min scan) were made during the middle of the day each day. On the 4th day the cage was baited with fruit during the observation period and on the 5th day the monkeys were given one of their daily feedings during the observation period in an attempt to increase social interactions.

For the following nine days, 10 min tests of pairs of animals were done. The animals were paired randomly, and four pairs were observed per day with different pairs each day - all possible combinations were tested from 2 to 5 times. Following this, three days of group observations were taken using the scan-focal-scan procedure to see if the paired exposures had generated any increase in enlisting behaviors. We next examined dyadic/triadic interactions for four days. In this procedure, a pair of animals was placed in the cage and observed for 10 min. A third animal was then introduced and the observations continued for another 10 min. One member of the original pair was then removed and the two remaining animals were observed for 10 min after which a new animal was introduced to form

another triad, and so on. Four triadic combinations were observed each day. This was followed by five days on which the "strange" male, Defeat, was introduced to pairs of C-Troop males. Defeat was introduced for 10 min following 10 min observation of each C-Troop pair. Three dyad/triad sets were observed each day.

Next, two days of scan-focal-scan observations of the entire group were followed by tests involving exposure to a different strange male. This male, Alabama, was an aggressive, fully adult male who was the second ranked male in I-Troop at the time. During the following 18 days C-Troop was tested with Alabama placed in a small cage outside the social test cage; observations of the reactions of both the entire group and of selected pairs were made. Then the small cage containing Alabama was placed inside the social test cage and group reactions recorded. Next, individual C-Troop males were observed with Alabama still in the small cage; this was followed by releasing Alabama in the social test cage with each of the C-Troop males separately. The series concluded by observing triads composed of selected pairs of C-Troop animals plus Alabama for 2 days and both Alabama and Defeat (introduced successively to each pair) for 3 days.

Then the C-Troop males were tested with additional strangers that were placed directly into the cage with them. Tests were conducted using individuals and pairs of C-Troop males. The strangers were males of intermediate rank and nonpregnant, nonnursing females that were taken from T-Troop only for the time it took to conduct these tests and then returned to their own group. Twenty days of tests were employed, during which there were four days on which no strangers were introduced in order to allow us to look at baseline interactions.

The results of all these observations were:

a. There was very little overt agonistic social behavior among the C-Troop males in the group situation, in pairs, or when triads of C-Troop members were observed. The most frequent agonistic behaviors were "lid", a low intensity and somewhat ambiguous aggressive behavior and "lipsmack", a low level submissive or appeasement behavior. There was virtually no "enlisting" behavior. Most social behavior involved sitting next to each other, grooming, and occasional hugging and mounting. Thus, simply keeping the animals in individual cages and bringing them together for just an hour or two a day did not induce an increase in the frequency or intensity of agonistic interactions. The five males formed a dominance hierarchy, but the social structure was apparent only when data from a good many days of observations were combined.

Such tests of social behavior are adequate for detecting overall increases in social behavior and increases in agonistic behavior, but would be of little use in studying CW-related agents that might produce decreases in social interactions and agonistic behaviors. The absence of enlisting behaviors in these situations was disappointing, since it was hoped that this behavior would provide a useful index of cooperation between animals.

b. Keeping the animals together as a group during several days did little to enhance the frequency or intensity of social interactions when the procedure of using brief daily exposures to each other was reinstated. Baiting the cage with fruit or feeding the animals during the observation period produced brief flurries of social activity, but these habituated rather quickly during an observation period. Such procedures do not appear to be very useful for experiments which would require repeated daily tests.

c. Exposure to the young adult male, Defeat, a monkey that had very low social rank in its former troop, produced little agonistic behavior. Exposure to Alabama, a high ranking male from I-Troop, did elicit agonistic behavior during early exposures when Alabama was caged inside the social cage and when he was free to interact directly with the C-Troop males. There was intense interest in the female strangers and this was accompanied by some agonistic activity. The three highest ranking C-Troop animals attacked the stranger males on a number of occasions; several fights had to be broken up by the observer, and both stranger and C-Troop males sustained some minor injuries during these tests. Habituation of agonistic behavior across days was rapid, however, and, once again, little enlisting behavior occurred. The results indicated that it would be necessary to keep changing stimulus animals in order to generate appreciable amounts of agonistic behavior in the laboratory tests of social behavior. Careful selection the stimulus animals would be necessary to minimize the potential for injuries. However, the procedure does work and it has a definite place in the behavioral test battery, although it would not be practical to use it on an everyday basis because of the habituation problem.

Prior to the last year of the project, a food pellet dispenser was installed in the social testing cage and the C-Troop monkeys were trained to take banana pellets delivered automatically on a VT-30 sec schedule. During the sessions, a cue light on the panel was illuminated, the light in the food hopper came on, and the pellet dispenser was activated on the

average of once every thirty seconds. The initial results of pair and group social testing during dispenser operation were very encouraging in that the monkeys increased their agonistic activities significantly in competing for access to the feeder. A comparison of mean daily frequencies of the various categories of social behavior over one week during which the feeder was turned off with one week of data with the feeder operating is given in Table 6.

Table 6

Mean Daily Frequencies of Social Behavior in C-Troop with Pellet Feeder On (5 days) and Off (5 days)

Behavior	Feeder Off	Feeder On
Submissive	2.0	7.0
Aggressive	1.7	8.0
Affiliative	86.7	71.5
Sexual	4.7	3.0
TOTAL SOCIAL	95.1	89.5

The next step was to shape the animals to press a lever for the banana pellets. They were then given individual training sessions until each had stabilized on a fixed ratio - 10 (FR 10) schedule of reinforcement which required the animal to make 10 responses for each pellet. Once each animal had achieved stable performance on this task, the animals were tested in pairs and as a group while the schedule was in effect. Later, the procedure was modified to include a shift to a more stringent schedule requirement during testing. This method involved placing the monkeys together and observing them for 5 - 10 min with the feeder off, using a cue light to signal the availability of food on the FR 10 schedule, and then raising the requirement to a FR 20 halfway through the test session. The results obtained with this procedure will be described in the section on cooperative behavior.

Cooperative behavior. One of the objectives of the project was to investigate ways of studying cooperative behavior. In our animals, cooperation occurs as a component of both affiliative and agonistic behaviors. Allogrooming and sexual behavior are obvious examples of interanimal cooperation and specific postures and gestures have evolved which are used to solicit and initiate these activities. They occur regularly

so that increases and decreases in response frequencies as the result of experimental manipulations can be assessed easily. Mutual grooming in which the animals take turns grooming each other is a marker which identifies a stable social relationship between individuals. The amount and direction of the various affiliative behaviors were recorded throughout the project and many examples of these data are presented in the report. A detailed analysis of affiliative behavior grouped by age, sex, and kinship classes was completed for all of the monkeys in T- and NT-Troops just prior to the initiation of the present project (Perkins, 1982). Data were collected for five months and each daily observation period involved two 20 min scans and eight 5 min focal obsevrations of selected monkeys. Among the findings in this study was that the troop members spent a majority of their time in affiliative interactions, but typically interacted with a relatively small number of other monkeys. In the adult males, which were the focus of most of the work in the present project, Perkins found that the greatest amount of nonsexual affiliative behavior involved grooming and sitting with adult females, interactions with other adult males were second and with male subadults third in frequency and duration. She also determined that the extent to which adult males interacted with other adult males depended upon the level of aggression among the adult males. In T-Troop, where aggression was low, the males spent almost as much time grooming each other as they did adult females; in NT-Troop, where the rate of aggression was almost four times greater than in T-Troop, intermale grooming was significantly lower. The overall results of the Perkins study were reconfirmed during the current project.

Another way in which the animals cooperate occurs during agonistic encounters. Some of this has been described in the sections on social organization and on C-Troop social behavior. An eyewitness account of a dramatic social upheaval in I-Troop is presented in the section of diazepam effects on social behavior. This provides a description of the behaviors involved in cooperative aggressive interactions. When several monkeys are involved in attacking one or more members of the group, it is not always clear that they are actually coordinating their activities. However, when enlisting (see the description in the section on C-Troop social behavior) results in the enlisted monkey aggressing against the target, it can be said that cooperation has occurred. One might expect that one of the functions of such agonistic alliances between monkeys would be to challenge and defeat dominant animals. However, in his discussion of multianimal agonistic interactions in captive cynomolgous macaques, de Waal (1977) noted that much of the aggression involving cooperation against a common opponent was directed down the dominance hierarchy and might be involved in reducing mutual tensions by redirecting them toward subordinate "scapegoats". He further suggested that many aggressive

alliances regulate relationships between allies rather than relationships with the opponent.

Although we were unsuccessful in generating enlisting behaviors in C-Troop, we have since found that moderate levels of enlisting can be induced under certain circumstances. After the present project terminated and it was no longer necessary to maintain the integrity of the social groups for behavioral performance testing, we conducted a series of introductions of unfamiliar males into I-Troop which by then had been reduced to four monkeys. Three adult males were introduced, one at a time, at intervals of about two weeks. Each introduction generated some enlisting behavior. Table 7 gives the frequencies of enlisting, cooperation (number of times the enlisted animal threatened the opponent) and aggressive, submissive and affiliative behaviors for each of the introductions. In gathering the data, the observers used a 20 min group scan on the original group before the new monkey was introduced after which another 40 min of scan data were recorded.

Table 7

Social Behavior Frequencies in I-Troop During Introductions of Adult Male Strangers

	Enlist	Cooperate	Aggressive	Submissive	Affiliative
1st Intro (Rhetoric)	15	9	132	79	32
2nd Intro (Lucifer)	8	6	148	61	14
3rd Intro (Horatio)	8	8	106	66	18

It seems likely that our original failure to induce measureable amounts of enlisting in C-Troop was due to the fact that the C-Troop animals did not live together all of the time and that the temporary associations were not long enough to establish the alliances needed to generate cooperative agonistic behavior. In I-Troop, such relationships had time to develop and introducing a stranger made them apparent. With one exception, all of the enlisting was done among the original members of the group against the newly introduced males. During the introduction of Rhetoric, all four original troop members were both enlisters and enlistees at least once. The alpha male was enlisted 7 times, the beta 6 times, the third ranked male 2 times, and the fourth, once. The second (6 times) and third ranked (6 times) animals did most of the enlisting. During Lucifer's introduction, all 8 instances involved enlisting of the alpha by the beta animal and 6 of the 8 instances during Horatio's introduction involved mutual enlisting by the alpha and beta males.

Thus, it appears possible to manipulate the social environment to generate agonistic cooperation. Such manipulations are not routine, however. Introducing stranger males to an established troop yielded much higher levels of agonistic behavior than that produced by the removal/replacement technique described earlier (e.g., Table 5). There were a number of minor wounds among both the resident and the introduced males. The fate of the introduced animals varied. Rhetoric began engaging in grooming bouts with the others after about two weeks and was accepted as the bottom ranked animal. Horatio began to assert himself after a few days and began working his way up the dominance hierarchy, which became unstable. Lucifer, who was unwounded, went into shock several hours after being introduced and could not be revived. The procedure entails a certain amount of risk and should be used in the test battery only when absolutely necessary to the objectives of the testing program.

Our attempts to develop an instrumental task to study active cooperation between monkeys were not successful. In part this was a function of the heavy testing schedule which reduced the amount of time that could be devoted to developing such a task. The first task selected for a pilot project involved trying to get the animals to pull a string to move a wheeled platform containing a food reward within reach of the monkey. Once animals learned to do this individually, we planned to run the string around a pulley such that, if both ends of the string were pulled at the same time, by two monkeys, the platform would move within reach. If only one monkey pulled, the string would spin around the pulley and the platform would not move. The literature (e.g. Mason and Hollis, 1962) suggested that the optimal conditions for a cooperative task of this kind would involve animals that had been housed together and thus had the opportunity to develop stable social relationships. We originally planned to use the C-Troop monkeys for this cooperative task, but were prevented from doing so by the other demands of the social testing schedule. We needed to continue the agonistic pair and group testing with C-Troop to meet other objectives of the project. Efforts to build an enclosure to house a pair of young adult females for the initial pilot work were thwarted by USDA inspectors who would not approve the wood and chain link caging that was to have served as both housing and test environment for the monkeys. The technician who had been assigned the development of the task then left the project. Because of the limited time left on the contract we were unable to replace him and schedule problems prevented assigning the problem to another graduate student. Whether we would ever have gotten enough monkeys to succeed on the task to provide a large enough sample for drug testing is problematical.

Although we failed to develop an instrumental cooperative task which would meet the criterion of showing that the monkeys would work together to complete a test involving the active performance of two or more individuals, the operant panel in C-Troop provided an alternative approach to the problem. Observers scoring the social behavior of the C-Troop males also recorded the identification of each animal pressing the lever. As noted earlier, introduction of the FR operant schedules to the C-Troop social testing produced an increase in agonistic behavior and some of this involved competition for access to the operant panel. After a few days, however, competition decreased and the monkeys began to "take turns" at the panel. There were several interesting aspects to this kind of social cooperation. First, there was a "normal order" of precedence in lever pressing. This was not strongly related to social dominance rank for, although a dominant monkey could always displace a subordinate, the alpha male was usually the third or fourth animal to operate the panel. Second, changeovers of the monkeys at the panel were characteristic of different dyadic relationships. We examined several thousand of these changeovers and found that they could be readily classified in three primary categories: "Nonsocial" changeovers were scored whenever an animal abondoned the lever and was replaced by another animal who approached the panel from a distance. "Agonistic" changeovers could be either "active", involving aggression directed toward the monkey at the panel, or "passsive", in which the monkey at the panel stopped pressing and showed submissive behavior toward an approaching monkey. "Cooperative" changeovers were rated "active" when they were accompanied by an exchange of affiliative behaviors - usually ventral-ventral hugging, sometimes accompanied by lipsmacking and without any agonistic components. This pattern was characteristic of the alpha male when he approached the panel. Typically he would approach, the other animal would lipsmack to him, he would lipsmack in return, they would exchange hugs, and the alpha male male would begin to barpress while the other animal moved off. "Passive" cooperative changeovers involved one animal approaching and sitting next to the one doing the lever pressing. He would wait until the other left the panel and then begin to press. We came upon this way of looking at "cooperation" late in the project and had not developed a way of automating the evaluation of the changeover data. Nevertheless, the preliminary analyses of the data, accomplished by examining the social behavioral sequences surrounding the changeovers, suggest that utilization of the changeover phenomena may provide a valuable addition to the test battery. A sample of the data, obtained from one of the diazepam experiments which is reported in detail in a later section, is presented in Table 8. The animals were tested over 7 placebo days and 7 diazepam days. The total number of changeovers was about equal for the two conditions, but there were fewer nonsocial changeovers on diazepam days. Most of the

difference was due to an increase in passive agonistic changeovers. Diazepam did not affect cooperative behavior.

Table 8
Active and Passive Cooperation, as Measured by Changeover Type in the Fourth C-Troop Diazepam Experiment.
(Data are proportions of the total changeover frequencies)

	Total Frequency	Non-Social	Changeover Type: Agonistic Active	Agonistic Passive	Cooperative Active	Cooperative Passive
.40 mg/kg Diazepam	126	.30	.11	.16	.17	.25
Placebo	130	.43	.11	.06	.15	.24

As noted above, the heavy committment to social testing in C-Troop did not permit more extensive work with an operant cooperative task. Because of our interest in the problem, we have continued to try to develop such a task. Recently, we installed a second operant panel in the C-Troop social cage. Both "competitive" tasks, in which one monkey of a pair can earn a reinforcer by completing the requirements of a schedule before the other, and "cooperative" tasks, in which they work to deliver reinforcers to each other, are being evaluated. Should the need arise to utilize the test battery in the future, there will be the potential for employing such tasks.

Drugs and Social Behavior:

Caffeine effects on social behavior. A study of the effects of caffeine sodium benzoate on social behavior was conducted with the adult males in T- and I-Troops. Intramuscular doses of 0.8, 4, 8, and 12 mg/kg were alternated with days on which the animals received injections of physiological saline. On each day, 3 of the 6 males in T-Troop received caffeine and the other half received saline. In I-Troop, the alpha male and the bottom ranking male received saline injections on all days; 3 of the remaining 6 males alternated with the other 3 males in receiving drug or placebo each day. The males were removed from their troops, weighed, injected, and returned to the compounds for observation of their social behavior using a combination of the 10 min scan - 5 min focal - 10 min scan procedure in effect during the first year of the project. The order in which the doses were administered was 4, 8, 0.8, and 12 mg/kg. Each male tested with drug received one administration of each dose.

Caffeine had no discernible effect on social behavior at any of the doses employed. Data from the group scan and focal

animal procedures were analyzed separately and in combination but did not reveal any consistent changes in behavior. A fight took place between the second and third ranked animals in T-Troop on the day they both received the 4 mg/kg dose, but it is highly unlikely that this was a drug effect. No changes in frequencies of agonistic social, "other" social, or nonsocial behaviors were seen in these, or any of the other animals, at any dose of caffeine. The results from I-Troop, in which the top and bottom ranked animals were not given caffeine, were essentially identical. There was no overall increase or decrease in social behavior and agonistic behavior, which was at a very low level on saline days, did not increase at any dose of caffeine sodium benzoate.

Group social behavior following injections of caffeine sodium benzoate also was examined in the five original C-Troop males following injections of caffeine sodium benzoate or atropine sulphate. The procedures and results of this experiment may be found in the section on atropine effects.

Atropine effects on social behavior. The first study on the effects of atropine on group social behavior was done with C-Troop. The protocol included intramuscular doses of 4, 12 and 36 mg/kg caffeine sodium benzoate as well as i.m. doses of .032, .08 (3 times to each monkey), and .20 (3 times to each monkey) mg/kg of atropine sulphate. The delay between the injection of the last animal and the beginning of testing was 5 min for all caffeine doses. Delays of 5, 30 and 60 min were used with the three administrations of the .08 and .20 mg/kg doses of atropine. Social tests were conducted five days a week, for five weeks. During the 25 days of testing, there were three days on which all animals received placebo and one day when neither drug nor placebo was given. Social observations began with a 5 min group scan followed by 5 min focal observations of each animal and concluded with another 5 min scan. For the first 16 days of the experiment, the alpha male always received a saline injection each day while the other four animals alternated drug and placebo days with two animals getting the drug each day. During the last nine days of the study, which included doses of 36 mg/kg caffeine and .032, .08 and .20 mg/kg atropine, the alpha male was also given the drug on alternate days. This experiment was followed by a pilot study in which doses of .08 (twice) and .20 (twice) mg/kg atropine methyl nitrate were used with the same behavioral observation procedures and a 30 min delay between injection and testing.

The results can be summarized by the statement that there were no effects of caffeine, atropine sulphate, or atropine methyl nitrate on social behavior at any dose or post injection delay. The C-Troop males exhibited very little agonistic behavior at any time - most of their interactions involved

grooming and playing. The outcome of this study pointed up the problems with the social testing procedures being employed with C-Troop and led to the series of behavioral studies of C-Troop described in an earlier section of this report.

The effects of atropine sulphate on social behavior in the eight I-Troop males was examined with doses of .032, .08 and .20 (twice) mg/kg and a delay of 30 min between injection and testing. The Mondays of each of the two weeks of the study were placebo days for all animals; the monkeys alternated drug and placebo days the rest of the time, with half of the animals receiving the drug each day. In this experiment there was a drug effect at the .08 and .20 doses. The frequency of agonistic behavior - both aggression and submission - was sharply reduced in the group on the days when some of the animals were given atropine. The mean frequency of agonistic encounters on the two days when all monkeys got the placebo was 19.0; on the days half the animals got atropine the mean was 5.0 for the four days some animals got .20 mg/kg, 6.0 for the two days some got .08 mg/kg, and 12.5 for the .032 mg/kg days. The effect appears to be specific to agonistic behavior and does not reflect a general depression of social activity since no consistent changes in frequencies of grooming behavior were observed. It appears likely that the failure to see any drug effect in the experiment with C-Troop was due to the low baseline levels of agonistic interactions in that group.

Diazepam and social behavior. The initial studies of diazepam effects on behavior involved studies of complex problem solving (T-Troop) and of performance on two different schedules of reinforcement - a variable interval schedule with I-Troop and a DRL schedule with the NT-Troop males. (The results of the experiments themselves are presented in later sections of this report.) Toward the end and shortly after these experiments were concluded there was a dramatic increase in agonistic behavior in the three troops. Several animals in NT-Troop, including the alpha male, had to be removed for treatment of injuries which occurred at this time. Similar, but less severe, problems were seen in T-Troop, while I-Troop underwent a major reorganization of its social rank structure as a result of the increased aggression. Gus, the alpha male, was successsfully challenged by Spiro, the second ranked animal. Three days after Spiro had established himself as the alpha, he was attacked by two intermediate ranked animals, Yuk and Yamamoto. A lengthy fight ensued with Spiro, Equal, Quotation and Cracker ranged against Yuk and Yamamoto and Gus cowering in a corner. At this point we intervened by removing the troop from its compound and housing its members in individual cages. They were kept apart for 24 days during which time a series of blood samples were obtained. When they were put back together, there was a moderate amount of aggression which was accompanied by substantial increases in levels of

plasma prolactin and cortisol (details of the hormone changes will be reported in the next section). The social behavior was "normal" in that patterns of aggression and submission were similar to those observed prior to the social upheaval. Spiro was the alpha, Gus was ranked second, and the other ranks were largely unchanged. Three weeks later, the I-Troop males were placed in a study of the effects of diazepam on performance in a shock suppression paradigm (see the section on operant performance for details). In this protocol, half the monkeys in the troop received a given dose of diazepam on a given day while the remainder got a placebo (vehicle); the next day the half that had received drug on the first day got placebo and the remaining animals were given that dose of diazepam. Toward the end of the experiment, on the second day of administration of a dose of 1.60 mg/kg of diazepam, another major altercation occurred. This took place in the group's indoor cage in the laboratory and began about five hours after the drug injections were given. Our laboratory technician, who was present from the beginning, reported the episode as follows:

> "I-Troop has been involved in a valium study to look at effects of valium on response suppression in the operant test. As with previous drug (diazepam-BNB) studies, a rank shakeup began during the two week long rep.. Spiro had been alpha prior to today. Upon return to Room 119, Spiro and Yamamoto sat on the perch, ventral-ventral hug position. This then led to grappling and biting between the two within about twenty seconds. Yamamoto clearly got the better of this encounter, as he displaced Spiro. Spiro broke off this interaction by jumping down to the floor, leaving Yamamoto on the perch alone. The other troop members avoided the perch and Yamamoto paced the length of the perch with arched tail (a dominance display-BNB). He was joined by Yuk, who engaged in much lip-smacking and attempts to groom Yamamoto. After several minutes of this, Yuk then threatened Spiro, all the while attempting to enlist Yamamoto. Yuk slapped Spiro, but seemed unable to displace him unless Yamamoto was in close physical proximity. at this point, all the other monkeys in the troop were avoiding any contact with any of the three participants.
>
> "I then left the room for about five minutes to notify Dr. Bunnell of the goings on. When I returned to the room, the situation had changed quite a bit.. Yuk and Yam had now been displaced by Spiro, et al. It appeared that Spiro had enlisted all the other I Troop members against Yamamoto and Yuk. Quote, Equal, Cracker and Spiro were now backing Yam and Yuk up against the wire. Spiro had backed Yuk into a corner on the floor, and was slapping him and pulling his tail quite vigorously. Yuk's only response to these attacks was a clearly submissive squeal. Yam was directly above Yuk on the wire, in the upper corner of the cage. He was fending off attacks from Cracker and was showing no interest in Yuk.

Yuk's attempts at enlisting Yam were either ignored by Yam, or perhaps Yam could not see them. Spiro, while attacking Yuk, would often look up at Yam and lip smack, then resume his attack. This action went on for about five minutes, not on a continuous basis, but with short respites during which time all I Troop members would threaten members of T and NT Troops (they were in the adjacent indoor cages-BNB).

"Quote then became more active in the fight, joining up with Spiro and Cracker. Equal then began to threaten with Quote, generally directed toward Yuk. Joined by these two, Spiro then was able to move Yuk down (the length of) the cage and away from Yam. This movement resulted in Yam moving down the length of the wire, maintaining his elevation (above the floor). Cracker then leapt from the perch to the wire and engaged Yam in grappling and biting. This resulted in Quote and Equal moving more behind Spiro, who then drove Yuk back to the other end of the cage. Yam attempted to follow on the wire. As he turned....., Cracker attacked from behind and beneath. He got Yam's leg in his mouth and bit for at least three seconds. As Yam pulled his leg free, I could see a lot of blood run, so I decided to intervene.....

"All of I Troop is now in room 114 (in individual cages-BNB) for the weekend, we will look at a social test rep. on Monday." (From a written report by T. L. Peacock, Research Technician II, dated 7 March, 1986.)

The incident appeared to be very similar to the one which had taken place two months earlier except it happened during, rather than shortly after the conclusion of, a diazepam/ performance experiment. On the day of the fight, Yamamoto, Quotation, and Equal had received the 1.60 mg/kg dose of diazepam while Spiro, Yuk, and Cracker, who had this dose of diazepam the day before, got injections of vehicle.

All of the upheavals within the social groups reported above took place in winter at a time when the weather was bad. Many cold, damp days prevented social observations in the outdoor compounds. It was not until the weather warmed and it became possible to schedule daily social testing that we were able to explore these apparent effects on social behavior in greater detail, although we did do some testing of diazepam effects on C-Troop pairs which will be described later in this section..

The first experiment on diazepam effects on group social behavior utilized seven I-Troop males. (Alabama was no longer in the group.) Drug and placebo doses were prepared and coded by the PI, administered by the lab technician, who did not know the code, and animals were tested by observers who did not participate in the drug injections although they were aware that a drug study was in progress. (This procedure was followed

throughout all of the drug studies.) Behavioral observations were started 15-30 min after the last monkey was injected (0815-0830 hours EDT). Two observers scored the behavior during the 60 mir tests. One observer started with a 30 min group scan and finished with a series of six 5 min focal observations (Gus was not included as a focal animal, see below). The other observer started with the focals and concluded with the 30 min scan; observers alternated starting the day with focals or a scan and the order of the focal observations was randomized within and across days. The monkeys were given i.m. doses of 0.16, 0.40, 0.80 (twice) and 1.60 mg/kg. The order in which the doses were administered was .80, 1.60, .16, .80 and .40 mg/kg. The experiment extended across 17 days, but no drugs were given and no social observations taken over the two weekends that occurred during that period. Mondays were placebo (vehicle) days for all animals; through the rest of the week, three monkeys received one of the diazepam doses each day while the rest were given vehicle. The drug and vehicle days were alternated so that monkeys receiving drug one day got vehicle the next day. Gus, now the fifth ranked male in the hierarchy, had been ill during the week prior to the experiment and he was not given any diazepam, but received vehicle every day. He was returned to the troop on day 1 of the experiment, when all monkeys received vehicle. Based upon the usual effects of such a manipulation (see the preceding section on social manipulations) we expected a general increase in agonistic behavior against which the drug effects could be studied.

Equal, Cracker, and Yamamoto alternated with Yuk, Spiro, and Quotation in receiving diazepam every other drug day. At the time of the experiment, the ranks in the social hierarchy were:

1. Equal
2. Yuk
3. Spiro
4. Quotation
5. Gus
6. Cracker
7. Yamamoto

The reintroduction of Gus (first vehicle day) produced a large amount of agonistic behavior - so much that the observations were extended for a second hour to monitor the activity. During the second hour, agonistic behavior declined by 60% and affiliative behavior increased by 25%. A comparison of the data generated by the scan and focal techniques indicated that the observers picked up more aggressive behaviors and fewer submissive behaviors using the focal procedure. The frequencies of submissive, aggressive, and affiliative behaviors for the two procedures during 115 minutes of observations were:

	Submissive	Aggressive	Affiliative
Group Scan	18	78	65
Focal Animal	7	105	56

If the introduction of Gus had generated a persistent increase in agonistic behavior, we could have expected to see heighted frequencies of agonistic behavior in the animals receiving vehicle for several days no matter what the effects of diazepam on social behavior. However, agonistic behavior fell to low levels very quickly in the animals receiving vehicle. Thus, it was not possible to use the data from the first day to compare to diazepam days and they have been omitted from the analyses which follow.

There was very little agonistic behavior during the course of the experiment. Table 9 gives the mean frequencies per monkey per day obtained from the group scan procedure. There was an increase in both aggressive and submissive behavior at the .40 mg/kg dose and an increase in submissive behavior at the .16 mg/kg dose. The small amount of submissive behavior seen at the 1.60 and .80 mg/kg doses came from Gus, who received vehicle on both days, and from the low ranking monkeys on the days they were given vehicle. The frequencies of affiliative social behavior were depressed at the highest dose and normal at the other three doses. Data from 11 days preceding the experiment are included in the Table.

Table 9

Mean Frequencies of Social Behavior per Monkey per Day by Diazepam Dose. Group Scan Data from I-Troop.

	Behavior Category		
	Submissive	Aggressive	Affiliative
Preexperimental	.09	.27	4.57
Postexperimental	.04	.08	2.50
Vehicle	.36	.07	5.00
.16 mg/kg	1.29	.07	5.00
.40 mg/kg	.71	.79	5.43
.80 mg/kg	.54	.11	5.46
1.60 mg/kg	.50	.00	3.07

There were some obvious differences in agonistic behavior between the pre- and postexperimental and the vehicle days. Only the last two vehicle days are included (see above) and it is possible there may have been some metabolite effects operating on the vehicle days. Comparing drug days with vehicle days yielded four reportable differences; the increase in aggression at the .40 mg/kg dose, the increases in submission at the .16 and .40 mg/kg doses, and the decrease in affiliative behavior at the 1.60 mg/kg dose. Analysis of the scan data for two days on which the 1.60 mg/kg dose was given showed that when the six monkeys were given diazepam they made only 5 affiliative responses while they made a total of 38 when they received vehicle. This was confirmed by the focal data in which drug and vehicle data could be directly compared in all six monkeys - the mean affiliative behavior per animal for 1.60 mg/kg diazepam was 1.14; for vehicle it was 3.67. The data are interpreted as reflecting the sedative effects of the drug at this relatively high dose. The monkeys moved around very little and also exhibited some mild ataxia.

Unfortunately, the focal observation technique picked up very little agonistic behavior - a total of 2 aggressive and 4 submissive behaviors exclusive of vehicle days - and contributed nothing the interpretation of agonistic activity. Analysis of the scan data produced some interesting details. Of the 11 aggressive behaviors recorded on the two days the monkeys received the .40 mg/kg dose of diazepam, none were by animals that got the drug that day and the largest number of aggressive behaviors occurred on the day the alpha male got diazepam. The increase in aggression by the intermediate ranking monkeys was directed toward the two bottom ranked animals that received drug on that day. This accounted for the increase in submissive behavior. None of the intermediate ranked monkeys directly challenged the alpha male. This suggests that, while diazepam may produce a decrease in aggresssion by an animal, it may also alter its behavior such that it no longer provides the same kind of cues to other members of the group. In the case of an alpha male, who "controls" the behavior of the other animals, a change in his behavior could lead to increases in agonistic behavior in the other animals and, under certain circumstances, to challenges of the alpha. However, if this were the case, i.e. that it is the vehicle animals that increase aggression because drugged monkeys provide different social cues than nondrugged monkeys, we might have expected to see increases in aggression at some of the other doses. The other effect, the increase in submissive behavior at the 0.16 dose, does appear to be a drug effect, since 10 of the 12 submissive behaviors recorded (excluding Gus) occurred in the animals when they were drugged. The increase in submission was not in response to increased aggression in the group as only 1 aggressive behavior was recorded in the two days of observations. Overall, the

experiment told us very lit e about diazepam effects on agonistic behavior because of the low control levels of aggression and perhaps also because of the procedural choice involving which animals got the drug on alternate days. Several additional experiments were conducted to explore the problem further.

The next study was done with the seven adult NT-Troop males in conjunction with an open field experiment (see the open field section of the report for these results). It was done concurrently with the I-Troop experiment described in the last section. All animals got either diazepam or vehicle on a given day. Social observations were made when the males were returned to the troop after completing testing in the open field. This meant that social behavior observations were begun 1 - 2 hours after the drugs were injected. There were four diazepam days on which the i.m. doses were .80, 1.60, .16, and .80 mg kg. The study lasted 12 days; days 1, 2, 4, 8, 10 and 12 were placebo (vehicle) days and days 3, 5, 9, and 11 were diazepam days (no tests were run on days 6, and 7). This schedule was similar to that used with these animals prior to the outbreak of aggression in NT-Troop the previous December. Table 10 compares the mean daily frequencies of submissive, aggressive, and affiliative social behaviors for the 4 diazepam, 6 placebo and 5 preexperimental baseline days. Data are presented both for the 7 males that were in the diazepam experiment and the remaining members of the troop that were scored.

This experiment differed from the Experiment with I-Troop described above in that the adult males all got the same dose of diazapam on the same day, the time between injection and observation was longer (1-2 hours vs 15-45 min), and a large number of non-drugged females and juveniles were part of the social environment. The results were also different. It can be seen that aggression by the adult males is elevated at all three doses of diazepam. If one combines the aggression data for all four diazapam days, one can show a statistically significant increase over both baseline and vehicle day scores. However, the more interesting result involves the appearance of an interaction which, because of the small number of cases and the frequent appearance of zero frequencies, does not meet the criterion probability but suggests a potentially important interaction between drug effects and social rank. On the day the animals got the .16 mg/kg dose, all of the aggressive behavior was by the three top ranked monkeys in the hierarchy; on the .80 mg kg days aggression was spread evenly across all ranks, and on the 1.60 mg/kg day, the top four ranks had a frequency of zero and all of the aggression was by the fifth and seventh ranked males. (Juveniles and females were more aggressive on this day also.)

Table 10

Mean Frequencies of Social Behaviors per Monkey per Day in NT-Troop During the Open Field/Diazepam Experiment.

	Behavior Category		
	Submissive	Aggressive	Affiliative
5 Day Baseline			
All Monkeys (n=24)	.42	.22	3.55
Adult Males (n=7)	.14	.40	1.97
Others (n=17)	.53	.25	4.85
6 Day Vehicle			
All Monkeys	.88	.42	4.89
Adult Males	.54	.49	2.90
Others	1.02	.39	5.49
4 Day Diazepam			
All Monkeys	.75	.41	4.02
Adult Males	.46	.79	4.57
Others	.87	.25	3.79
.16 mg/kg Diazepam			
All Monkeys	.54	.29	3.04
Adult Males	.43	.71	3.71
Others	.59	.12	2.76
.80 mg/kg Diazepam			
All Monkeys	.73	.29	3.81
Adult Males	.71	.64	4.07
Others	.74	.15	3.47
1.60 mg/kg Diazepam			
All Monkeys	1.00	.75	5.42
Adult Males	.00	1.14	5.29
Others	1.41	.59	5.47

Aggression by high rank males was directed toward low rank males and juveniles; aggression by low rank males involved other low rank males and juveniles. No low rank male challenged an animal above him in the hierarchy. Possible drug induced changes in the frequencies of submissive behaviors are obscured by the vehicle induced increases over baseline, although the increase at .80 mg/kg can probably be interpreted as a being the result of the general increase in aggression by members of the male hierarchy. Finally, there was a dose dependent increase in affiliative social interactions. This differed from the I-Troop results and we examined the nature and direction of the affiliative behavior in some detail. There was a small increase in allogrooming under .16 mg/kg diazepam which levelled off at the two highest doses; the additional increases in affiliative behavior were due to increases in sitting with and hugging other aninals. The seven adult males directed .04 of their total affiliative behavior toward each other on vehicle days and .13 of their total on diazepam days. This small increase is not significant, so we conclude that the pattern of these interactions is essentially normal. Had the males showed a larger increase in the frequency of their contacts with each other, this might have contributed to increases in aggression, but this hypothesis was not supported by the data.

We were encouraged by the results obtained in the first NT-Troop experiment and by the suggestion that there might be an important interaction between social rank and dose. We next went back to the experimental paradigm we used in the initial study with I-Troop, but this time we gave diazepam to high ranking NT-Troop males on one day and low ranked animals the next. This study, which was begun 10 days after the one reported in the preceding section, utilized an additional dose of .40 mg/kg of diazepam; the order of administration of the doses was .80, .16, .40 and 1.60 mg/kg. On day 1 and day 8, all animals received placebo; on day 2, the three highest ranking monkeys in the adult male hierarchy got the .80 mg/kg dose of diazepam and the four lowest ranking animals received placebo; on day 3, the animals that received drug on day 2 were given placebo and vice versa; this procedure of giving either the lowest or the highest ranked monkeys diazepam on any given day was continued throughout the schedule. No animal received diazepam on two consecutive days and no tests were run on days 6 and 7 of the 12 day study. Social observations began 15 min after the seventh animal received its daily injection of either drug or placebo. Two observers collected data - one did a continuous group scan while the other used the focal animal procedure.

In Table 11, the mean frequencies per animal per day of behaviors in three social categories are given for five days before the experiment began, the two days on which all 7 adult males received placebo, the four days the 3 highest ranking males got diazepam and the four days in which the 4 lowest ranking males got the drug. Data are presented for the high rank (Hi) and low (Lo) rank males, and the other 17 animals ("Rest") scored during the group scans.

Table 11

Mean Frequencies per Monkey per Day of Social Behaviors in NT-Troop During the Social/Diazepam Experiment.

	Behavior Category								
	Submissive			Aggressive			Affiliative		
Rank:	Hi	Lo	Rest	Hi	Lo	Rest	Hi	Lo	Rest
n =	3	4	17	3	4	17	3	4	17
Mean 5 Day Baseline:	.33	.85	.84	1.27	.25	.33	3.01	2.65	2.47
Mean 2 Day Vehicle:	.50	2.25	1.71	2.50	1.63	.65	2.67	1.63	2.97
0.16 mg/kg									
To 3 High:	.33	1.25	1.41	1.00	1.00	.53	10.00	2.75	3.23
To 4 Low:	.00	1.75	.71	.67	2.75	.00	2.00	.00	.65
0.40 mg/kg									
To 3 High:	.00	.00	.76	.00	1.00	.12	3.67	2.25	5.12
To 4 Low:	.00	1.25	1.59	1.33	.50	.71	3.33	2.50	3.00
0.80 mg/kg									
To 3 High:	.33	.50	2.94	1.33	1.25	.18	2.67	3.00	2.35
To 4 Low:	.00	4.00	1.12	3.00	.75	.24	1.67	2.75	3.53
1.60 mg/kg									
To 3 High:	.33	.75	1.00	.00	2.25	.41	6.33	6.25	5.59
To 4 Low:	.33	1.75	.82	1.33	1.25	.47	2.33	5.25	6.47

Once again, the group scan data indicated that there was an increase in agonistic behavior on placebo days over baseline days. Using these vehicle days for our comparisons, giving diazepam to the high rank monkeys reduced their aggression; giving it to low rank animals produced a nonsignificant increase in aggression at the .16 mg/kg dose and reduced it at the other three doses. Aggression was slightly above placebo levels in the high rank animals on the day the low rank animals were given the .80 mg/kg dose and in the low rank animals on the day the high rank animals got the 1.60 mg/kg dose. Giving the .16 mg/kg dose of diazepam to the high rank monkeys increased their affiliative social behavior by a factor of 4, while the same dose to the low rank males, who showed more aggression at this dose, produced zero affiliative interactions by these animals. Affiliative behavior by high rank males declined at the .40 and .80 mg/kg doses and increased to baseline levels in the low rank males at these doses. The 1.60 mg/kg dose produced high frequencies of affliative behaviors throughout the troop when it was given to the high rank males; administering this dose to the low rank males increased these behaviors in the low rank males and the rest of the troop, but not in the high rank males. When increases in affiliative behavior occurred in the study, the nature of the affiliative behavior changed. At the .16 mg/kg dose, allogrooming increased in approximately the same ratio as all categories of affiliative behavior; with the three highest doses, however, allogrooming virtually disappeared in the drugged animals and was present at control or lightly elevated levels in nondrugged males, females and juveniles. Thus, the high levels of affiliative behavior involved mostly approaching and sitting with one another and hugging and relatively little mutual grooming.

In Table 12, the aggression data are examined from a different perspective. In generating this table, the focal observation data were used to identify the targets of the aggression, i.e, the frequency of aggression toward other males in the hierarchy vs that directed toward females and juveniles ("Rest"). Each data point represents the mean frequency of aggressive behaviors for the seven monkeys on the days they received each dose of the drug. The Placebo data are the means of the two days when all males received vehicle only. The "Rest" column refers to the females and juveniles who received neither drug nor placebo, but who were present when the drugs were given to the males.

Table 12

Mean Frequencies per Monkey per Day of Aggressive Behaviors in NT-Troop for Different Doses of Diazepam.

Aggression by:	High Rank Males		Low Rank Males		Rest	
Directed toward:	Males	Rest	Males	Rest	Males	Rest
Placebo	.33	4.67	1.25	2.00	.35	.94
.16 mg/kg	.00	1.67	2.00	1.75	.12	.41
.40 mg/kg	.33	1.00	.25	1.25	.00	.82
.80 mg/kg	2.67	1.67	.75	1.25	.06	.35
1.60 mg/kg	1.33	.00	1.75	1.75	0.00	.88

On placebo days, the high ranking monkeys directed most of their aggression toward females and juveniles while the low rank males divided their aggressive behavior fairly evenly between males and juveniles. (The low rank males were aggressive toward other low rank males, but not toward high rank males). The small increase in aggression by low rank males recorded in the group scans (Table 11) at the .16 mg/kg dose was also detected by the focal procedure which indicated that it involved increased intermale aggression. Similarly, the increased aggression by the high rank males at the .80 mg/kg dose was directd primarily toward the other adult males and secondarily toward juvenile and subadult males. The values in Tables 11 and 12 differ from each other to some degree because the former was generated from scan data and the latter from focal observations. The tables are included not only because they suggest an interaction between social rank and drug effects, but because they illustrate an application of the procedures for gathering and analyzing social data in studying psychopharmacological manipulations.

During the winter of 1987, shortly after the contract ended, we had an opportunity to try to recreate the social upheaval results from the preceding year under more controlled conditions. Using the six remaining I-Troop males, we gave all monkeys .40 mg/kg i.m. each day for 3 consecutive days; for the next three days they got either no injection (2 days) or vehicle (1 day); this was followed by 3 more days of diazepam, 1 vehicle day, and 2 no injection days. Weather prevented our gathering social data on one no injection day in the middle of the experiment and 1 diazepam day during the second set of 3 drug days, (but the drug was given). The scan data are summarized in Table 13.

Table 13

Mean Frequencies of Social Behaviors per Monkey per Day During the I-Troop Chronic Diazepam Study (.40 mg/kg)

	Behavior Category		
	Submissive	Aggressive	Affiliative
Three Day Predrug	1.33	.46	9.30
Three Day Diazepam-1	.28	.36	11.06
Three Day Post Drug-1	.67	4.05	6.83
Three Day Diazepam-2	.17	.13	11.42
Three Day Post Drug-2	.45	.45	8.70

The sharp increase in aggression during the three days following the first course of 3 diazepam injections (Post Drug-1) was significant. The one way ANOVA yielded a $F_{4,20} = 5.63$, $p < .02$. Posthoc Tukey tests showed that the only significant differences were between this period and the other four periods. (The small decreases in aggression during the two drug administrations were not significant.) An analysis of the scores for affiliative behavior produced a $F_{4,20} = 4.97$, $p < .01$. The significant differences by the Tukey test were between the two diazepam scores and the Post Drug-1 scores. Because of the high aggression on the Post Drug-1 days, affiliative behavior was reduced during that period and so it is not possible to conclude that diazepam produces increases in affiliative behavior despite the consistent trend in that direction. These results will be discussed further in the section which describes the results of a similar experiment with the C-Troop males.

Several experiments on the effects of diazepam on social behavior were conducted using the six C-Troop males. In the first study, which was done during the winter preceding the experiments with NT- and I-Troops described above, the monkeys were observed in the indoor social cage with the operant feeder activated on the VT 30-sec schedule. The drug dose was .80 mg/kg i.m. and the diazepam vehicle was used as the control. All possible combinations of pairs were run twice - making a total of 30 pairs. For each pair, the drug was given to the

dominant monkey on one test and to the subordinate monkey on the other test. Pairings were adjusted to avoid giving diazepam to a monkey on two consecutive days. Three pairs were observed each day, so each monkey was tested only once a day, and each test lasted 20 min. The results showed that this dose of diazepam reduced affiliative social behaviors such as grooming, but did not affect the frequency of submissive behaviors. Diazepam eliminated aggressive behavior in the dominant animals, but the baseline level was so low we cannot be sure that the effect was real. The frequencies of four classes of behaviors are given in Table 14. The Pre Drug and Post Drug columns in the table are data from all possible pairs obtained two weeks before and two weeks after the drug study.

Table 14

Diazepam and Social Behavior in Dyadic Interactions
C-Troop: 6 Monkeys in 15 Pair Combinations

Behavior	Pre Drug	.80 mg/kg Diazepam to Dominant	.80 mg/kg Diazepam to Subordinate	Post Drug
Submissive	8	12	16	15
Aggressive	4	0	3	3
Affiliative	63	38	45	75
Sexual	2	2	2	7
Total Social Behavior	77	52	66	100

The next experiment essentially repeated the preceding study, but used doses of .40 and .16 mg/kg of diazepam. Other differences from the first C-Troop study included giving the drug to selected pairs instead of all possible pair combinations and having the monkeys working on a FR 10 operant schedule for food reinforcement during the observation periods. (The monkeys received a food pellet for every 10 lever presses.)

The introduction of the FR 10 operant schedule initially produced a considerable elevation of agonistic behaviors. When the animals were observed as a group for four consecutive days the mean frequency of submissive behavior per monkey per day was 2.0 and the mean for aggression was 2.2. When the monkeys were tested in pairs before the diazepam study began (all possible pair combinations over 5 days), submission frequencies remained high, mean of 1.6, but aggression virtually disappeared, the mean was 0.07, and the mean frequency of affiliative social behavior recorded per monkey per day dropped

from 6.6 to 2.4. While comparing group and pair data is a bit like comparing apples and oranges, the amount of observation time was roughly equal for the two procedures and the reduction in aggressive behavior in the pair situation was disappointing.

We had planned to run the two doses over successive weeks, using the same 15 pairings with each dose. However, we had to replace the observer with a new person partway through the protocol. The .40 mg/kg dose was tested first (data were available for 11 pairings - the old observer was out on the day the last three pairs were to be tested and data from one pair were lost due to an equipment malfunction - and the .16 mg/kg dose was tested 8 weeks later (data were available for 14 pairings - one was lost due to equipment malfunction). Prior to each set of tests, all possible pair combinations were tested once. Malfunctions of the data recorder cost us the data from three pairs; thus, the preexperimental data contain 14 pairings for .40 mg/kg dose and 13 pairings for the .16 mg/kg dose of diazepam. Because of the change in observers and the delay between the administration of the two doses, the data were treated separately. Table 15 presents the data for the preexperimental pair combinations and the drug data for the two doses of diazepam.

Table 15

Diazepam and Social Behavior in Dyadic Interactions
C-Troop: 6 Monkeys in Selected Pair Combinations
(Data are Mean frequencies per Pairing)

	Submission	Aggression	Affiliative
.40 mg/kg Diazepam			
Preexperimental: (14/15 Pairs)	3.50	.14	5.20
To Dominant: (5 Pairs)	.80	.00	6.40
To Subordinate: (6 pairs)	.33	.00	7.67
.16 mg/kg Diazepam			
Preexperimental: (13/15 Pairs)	.53	.08	3.07
To Dominant: (8 Pairs)	.75	.00	9.00
To Subordinate: (6 Pairs)	.16	.00	8.67

As in the first study on dyadic interactions, there is too little aggression in the preexperimental pairings to conclude that diazepam reduced aggression. While there was a precipitous drop in submissive behavior between the preexperimental pairings and the .40 mg/kg days, levels of submissive behavior did not recover prior to the second half of the study. Either the drug effect of the .40 dose was an artifact related to habituation to the operant task or the drug produced a long term suppression of submissive behavior. The small increase in affiliative behavior at the .40 mg/kg dose was not consistent across animals; however, the within subjects ANOVA of the .16 mg/kg data yielded a significant $F_{2,10} = 15.10$, $p < .001$. Tukey tests showed the differences between preexperimental and both sets of pairings to be significant.

Three studies on the effects of diazepam on group social behavior were conducted with the C-Troop males. The first of these involved giving .80 mg/kg diazepam to three monkeys each day while the other three received vehicle. Tests lasted 50 min and began with a 10 min group scan, followed by one 5 min focal on each animal, and concluded with another 10 min scan. The animals had been trained to press the lever on the operant panel and a FR 10 reinforcement schedule was in effect during these sessions, i.e., a food pellet was delivered after every 10 responses. The experiment lasted 9 days, with the first day being a placebo day for all animals. On each subsequent day, various sets of 3 monkeys were given the drug until all 6 had gotten four administrations of diazepam. The schedule was arranged so that a different combination of 3 animals got the drug each day. (As it turned out, two monkeys (the 5th and 6th ranked in the dominance hierarchy) got diazepam on two consecutive days, while the other always went a minimum of 48 hours between drug administrations. Table 16 compares the social behavior of the group during the eight days that diazepam was given with six preexperimental days during which neither drug nor placebo were administered.

Table 16

Effects of .80 mg/kg Diazepam on Group Social Behavior in C-Troop (Data are Responses per Monkey per Day in Each Behavior Category)

	Submissive	Aggressive	Affiliative
Preexperimental	1.78	2.17	7.31
Experimental	.90	.60	7.73

The table allows a simple comparison of group social behavior during a period when the monkeys were not receiving either drug or placebo with a period during which some were getting diazepam and some placebo each day. There was a sharp decrease in aggression during the experimental period and a decrease in submission that was roughly proportional to the decrease in aggression. We found that the total frequency of aggressive responses recorded from both scan and focal observations during the eight drug days was only 29 compared with 78 from the six preexperimental days. When the daily scores were examined in more detail, it was determined that 24/29 of these were attributable to the alpha male, Rasputin, on the 4 days he received vehicle; he made no aggressive responses on the 4 days he recieved diazepam. The other 5 aggressive responses were made by the second ranked animal, Tolstoy; only one of these occurred on a drug day. On the preexperimental days, 5 of the 6 monkeys made at least one aggressive response, and 3 made 20 or more. We concluded that the .80 mg/kg dose reduced aggression; reduced submission, probably because of the decrease in aggression, and had no effect on affiliative behavior. In terms of procedural matters, the use of the FR 10 schedule caused some competition among the animals and produced higher levels of aggression during preexperimental observations than we had seem for some time, enabling us to observe the decrease in aggression with diazepam. On the other hand, the daily 5 min focal observations of each monkey produced disappointing results in that they picked up totals of only 8 agonistic behaviors and 77 affiliative behaviors across eight days. It appears that the total focal observation time for each monkey will have to be increased considerably to be sure of obtaining enough data for a meaningful analysis.

In the next experiment, a dose of .40 mg/kg diazepam was used with all animals getting diazepam on the same day. Drug days and placebo days were alternated until the monkeys had received 6 drug days and 7 vehicle days. For purposes of comparison with earlier work, there was a 7th drug day on which the animals were given a .80 mg/kg dose of diazepam (this took place on day 4 of the experiment). The FR 10 operant schedule was in effect beginning 5 min after the start of the observation period. After 20 min, the panel was turned off for 5 min after which it was reactivated and a more stringent FR 20 schedule was used for the last 20 minutes of the test. The purpose of shifting the schedule requirement was to try to increase competition for access to the lever. Group scan procedures were used throughout the sessions. Table 17 gives the means for placebo and .40 mg/kg diazepam days.

Table 17
Effects of .40 mg/kg Diazepam on Social Behavior in C-Troop
(Mean Frequencies per Day in Group +/- SEM)

	Submissive	Aggressive	Affiliative	Food Pellets Obtained
Vehicle (7 days)	5.14 (1.14)	4.71 (1.69)	65.86 (9.78)	180.29 (13.63)
Diazpam (6 days)	8.67 (1.76)	9.67 (3.43)	60.00 (8.30)	210.17 (12.30)
.80 mg/kg (1 day)	13.00	10.00	40.00	180.00

None of the differences between means of the placebo and .40 mg/kg days were statistically significant. In contrast to the preceding experiment where half of the monkeys were given .80 mg/kg each day, alternating days on which either placebo or .40 mg/kg of drug was given to all animals produced very different patterns of social activity in the group. The range of aggressive behavior scores was 0 - 12 on placebo days and 0 - 22 on diazepam days. The large increase in variance under diazepam prompted us to examine each aggressive interaction that occurred during the entire experiment. Throughout the study, the aggressive behavior was evenly distributed across ranks on the placebo days. The same was true on the first diazepam day, but during the middle of the study most of the aggression was between low ranking animals. During the last two diazepam days, when aggression was greatest (22 and 16 behaviors, respectively), aggression again became general and involved all 6 monkeys.

The third study on the effects of diazepam on group social behavior in C-Troop used repeated doses of .40 mg/kg diazepam and lasted 14 consecutive days. The first day was a placebo day; this was followed by three consecutive diazepam days, a placebo day, two days on which the monkeys were observed but neither drug nor vehicle were given; three more diazepam days, two placebo days, and two more days with neither drug nor vehicle. The FR 20 operant schedule was in effect during the observation periods. There was some submissive behavior, but only one agressive behavior was recorded during the first 7 days of observations. On the 9th and 10th days - the 5th and 6th diazepam days - there were 15 and 16 aggressive behaviors, respectively. On 11th and 12th days - the last two placebo days - aggression dropped to 12 and then 5 instances, and on the 13th and 14th days - when neither diazepam nor vehicle were given, aggression scores were zero. The data are summarized for the first and second week in Table 18. Placebo and no injection days have been combined in the "Control" column.

Table 18

Repeated Doses (.40 mg/kg) of Diazepam and Social Behavior in C-Troop (Mean Frequencies per Day in Group +/- SEM)

	Submissive		Aggressive		Affiliative		Food Pellets Obtained	
	Control	Drug	Control	Drug	Control	Drug	Control	Drug
Week One	1.1 (1.0)	1.7 (1.2)	0.1 (0.0)	0.4 (0.3)	53.0 (6.1)	54.3 (3.9)	106.0 (7.6)	120.0 (12.3)
Week Two	4.4 (2.2)	3.8 (2.0)	4.4 (2.8)	10.8 (4.8)	55.5 (5.5)	55.0 (3.6	121.5 (18.7)	150.3 (16.0)

A 2 x 2 ANOVA (Weeks by Drug), using a square root transformation of the aggression scores because of the large amount of variability, yielded a significant Weeks effect ($F_{1,7} = 7.21$, $p < .05$). Neither the Drug condition nor the interaction were significant. As noted above, aggression increased dramatically on the last two days of diazepam administraton during the second week, then tapered off across the two subsequent placebo days to zero on the last two days when no injections of either drug or placebo were given. Examination of the agonistic interactions on the last two drug days and the last two placebo days showed that the patterns of behavior were quite different. Normally, the frequency of submissive behavior recorded during a given day is equal to or greater than the frequency of aggressive behavior. This is because a monkey may make more than one submissive response to a single aggressive behavior by an opponent and because a submissive animal may avoid or grimace to a dominant opponent in the absence of any overt aggressive behavior by the higher ranking male. Table 19 gives the ratio of submissive to aggressive behaviors for the control (placebo and no injection) and diazepam days for the two weeks of the study. The Table shows that the high levels of aggression on the diazepam days of the second week were not accompanied by proportionate increases in submission. In contrast, the four days following showed a gradual return to a more normal pattern, i.e. 3:4; 7:5; 1:0 and 0:0.

Table 19

Ratios of Suomissive Behaviors to Aggressive Behaviors During Two Weeks of .40 mg/kg Diazepam Injections in C-Troop (Submissive:Aggressive)

	Control	Diazepam
Week One:	3:0	5:1
Week Two:	1:1	11:32

None of the other behaviors differed between control and drug days or between weeks. It is interesting that affiliative behavior was not affected at all, despite the increase in aggression during the second week. The nonsignificant increase in successful responding on the FR 20 schedule was examined further by looking at the number of food pellets received as a function of the number of times the animals started working on the schedule. During the first week, the monkeys completed 93% of the schedules initiated on both control and drug days. During the second week animals completed 93% of the mean of 129.3 schedules initiated on control days, while on the last three diazepam days they completed 88% but initiated a mean of 170.0 schedules. Thus lever pressing was interrupted more often, but the animals compensated by initiating more episodes of lever pressing.

This lengthy description of the studies of diazepam effects on social behavior has been included both because some of the data are interesting in their own right and because they illustrate some of problems and issues involved in trying to evaluate drug effects on complex social situations. For more than 20 years, the benzodiazepines have commonly been considered to have anti-aggressive effects, but this interpretation ignores both the complexity of the aggression concept and the studies which have found increases in aggression in some species and some test situations (see the review by Rodgers and Waters, 1985). In a study which has many parallels with our work with C-Troop, Delgado, et al (1975) called attention to the differential sensitivity of dominant and submissive rhesus monkeys to diazepam and to the importance of the social context for interpreting drug effects. We found increases, decreases, and no effect on agonistic behavior and these varied as a function of social status, social context (e.g., pairs, groups of adult males, or groups containing additional age/sex classes), stability of the social organization, dose, acute vs repeated doses, and whether or not all males in a study were drugged on the same day. Probably anxiolytic, sedative and other effects of diazepam operate in the social situation and their net effect is to alter the social signals and/or the perception of such signals which normally maintain relationships among individual monkeys in the group and protect the structural integrity of the group as a whole. A next step would be test caffeine, and other drugs, for their antagonist potential in an effort to delineate specific pharmacological mechanisms for the effects observed.

Clearly, the potential interactions between a drug, a behavioral protocol and social variables make it essential to administer a variety of drug doses in different protocols throughout a range of social contexts before drawing conclusions about the effects of that drug on social behavior and on performance in social situations.

D. Open Field Testing:

Open field testing was conducted to study the monkeys' willingness to enter a strange environment, their locomotor exploration in that environment, and their responses to novel stimuli placed in the field during testing. Earlier work with this test situation (see Bunnell, 1982) showed a relationship between scores in the open field and social behavior during initial, but not subsequent, exposure to the situation.

Testing was conducted in a square open field, 3.7 m on a side and 1.8 m high, located in a large room in the laboratory building. Walls and floor were painted white, and the floor was divided into 16 squares by a painted grid. Five threaded studs, one in the center and the other four arranged in a square pattern equidistant from the center and the walls, were imbedded in the floor. These were used to attach the novel objects used as stimuli in some of the tests. The open field was covered by chain link fencing and illuminated by four 150 watt floodlights placed above this ceiling. Two guillotine doors located at diagonally opposite corners of the arena provided the means by which animals could be introduced into the field. An elevated platform located along one wall outside the arena was used for observing and scoring behavior. Opaque curtains and a one way window prevented the monkeys from seeing the observers during testing.

Monkeys being tested were brought to the open field in transport cages; these cages were placed outside one of the guillotine doors for 5 min before the door was opened and the animal allowed access to the field. In a typical test, the animal was allowed 5 min to emerge into the field. (On some tests, if this time was exceeded, the animal was gently forced into the field and the test continued). "Emergence" required the animal to enter the arena and move beyond the first square in the field (a distance of approximately 1 m). When the animal emerged, the guillotine door was closed behind it and its behavior during the ensuing 5 min recorded by the observers. At the end of 5 min, the guillotine door was reopened and the monkey allowed to return to its transport cage. When the animals were tested in the bare field, without novel objects being present, the following measures were taken:

(1) Head Out Latency: Time from opening the guillotine door until the animal put its head through the door into the arena.

(2) Body Out Latency: Time from opening the guillotine door until the animal entered the square of the arena directly in front of the guillotine door.

(3) Number of Returns: Number of times the monkey returned to the transport cage after entering the first square ("body out").

(4) Emergence Latency: Time from opening the guillotine door until the animal "emerged" as defined above.

(5) Exploratory Moves: Number of squares traversed by the animal during the 5 min following its emergence into the field.*

(6) Return Latency: Time from reopening of the door following the 5 min exploratory period until the animal reentered its transport cage.

(7) Return Moves: Number of squares traversed during the return latency period.*

* Time spent on the floor is differentiated from that spent moving about on the ceiling during these periods.)

When novel objects were present in the arena, the frequencies of occurrence of the following additional behaviors were also recorded:

(8) Lip Smacking
(9) Orientation toward object(s)
(10) Manipulation of object(s)
(11) Threats toward object(s)
(12) Bites (object)
(13) Other contacts with object(s)
(14) Vocalizations
(15) Self directed behaviors (groom, masturbate, etc.)

General Findings

Shortly after the project began the eight adult males in I-Troop were tested using both the bare open field and the field with novel stimuli in place. Although the the tests were conducted primarily to train observers in the testing procedures, the nine days of observations produced useful baseline information on these animals. During the winter of 1984, the I-Troop males were retested in order to examine the stability of their responses across time. They were given 3 days exposure to the bare field followed by 2 days with novel objects present. Six males from T-Troop and 8 from NT-Troop were also tested at this time under the same schedule of 3 days of empty field followed by 2 days with a novel object in the field. During both the December and February tests on I-Troop and the February tests with NT-Troop, blood samples for plasma hormone assays were collected in conjunction with the open field tests. In conducting these tests, the guillotine door into the field was opened 1 min after the transport cage was put into position and the animals were allowed 15 min to emerge and begin exploration. The test was terminated if the animal had not emerged within the 15 min latency period.

The tests with I-Troop showed that the amount of locomotor activity was quite stable within individuals across the two tests which were separated by 66 days. Introducing the novel objects depressed activity on the first day in December, but had no obvious effect in March (the same objects were used). There was a high positive correlation (rho = +.86) between amount of activity and social rank in the male dominance hierarchy. At the time the tests were made, however, the observers were still learning to score social behavior and there were not enough reliable data to allow us to do a detailed analysis of the relationships between activity and the various categories of social behavior. In the December tests, the 7th ranked animal never voluntarily entered the open field, the 8th ranked animal did not enter on either novel object day and the 6th ranked animal did not enter on one novel object day. In the March tests, the 7th ranked monkey did not enter on one bare field and one novel object day. All other animals entered on all days. A relationship between rank and activity was seen in the 4 T-Troop males that voluntarily emerged on each day of testing. Unfortunately, the second and third ranked animals did not emerge within the 15 min criterion period on most days. In NT-Troop, however, there was no relationship between rank and activity. The second ranked animal did not emerge on any day, however, and the third ranked animal was ill and could not participate in the tests. When these animals were retested in the summmer, The two top ranked monkeys were the least active and the third ranked was the most active. Thus, the relationship found in I-Troop did not hold for the NT-Troop males. In the animals that emerged consistently, high ranking monkeys tended to emerge more quickly than low ranking animals in all of the troops; however, emergence failures by animals of various ranks obscured this relationship.

In March and again in May, 1984, the five original members of C-Troop were given a single exposure to the open field with a novel object present. A five min delay was introduced between positioning the transport cage next to the field and opening the door to allow access to the field. All monkeys voluntarily entered the field on both days and the maximum emergence latency was 7 sec. Three baseline blood samples and one postexposure sample were collected each time. The tests were run before the animals were reunited as a social group, so no comparisons of social data with open field data could be made. On the second test, the amount of activity dropped sharply in three monkeys, stayed the same in one, and increased in one. Interactions with the novel object declined from a mean of 8.2 to 1.2 across the tests (The same "novel" object was used in both tests.) Plasma beta-endorphin levels increased in 3 of 5 monkeys after exposure to the open field on the first test. Plasma from the second test was assayed for prolactin and cortisol; the results are reported in the section on stress hormones.

Analyses of the baseline data from the first three troops indicated that if emergence latencies were longer than 5 min, the monkeys were unlikely to voluntarily enter the field at all. On the other hand, the C-Troop data suggested that increasing the delay between positioning the transport cage containing the monkey next to the field and opening the door into the field from 1 min to 5 min reduced emergence latencies and increased the probability of a voluntary entry. As a result, the testing procedures were changed for the first drug study to incorporate a 5 min delay and a 5 min maximum emergence latency period following which the monkey was to be gently forced into the field. The latter procedure had the advantage of reducing the possible variability of the time between drug administration and begining behavioral observations in the field from 15 to 5 min. Utilization of the new procedure worked well on the control days of the first caffeine study and it was continued in the later studies with atropine and diazepam.

The initial work also indicated that responses to a particular stimulus object habituated rather quickly across days. It was determined that it would be best if the monkeys were not exposed to the same object more than once during any six month period and that completely unfamiliar stimuli should be used wherever possible. The preliminary work had used 3-5 stimuli, placed in various locations in the field, per test. This was reduced to one object, located in the center position of the field, for all of the drug testing. We had found no advantage to using several objects at once and the use of one object per test reduced the number of novel objects needed during each experiment.

Drug Studies with the Open Field

Effects of caffeine on open field behavior. An experiment on the effects of caffeine on behavior in the open field was conducted with the seven adult NT-Troop males. In this study, the animal was given 5 min to enter the field after the door was opened. If it failed to do so, it was gently pushed into the arena and the test continued with the usual procedures. Caffeine sodium benzoate or control injections (physiological saline) were given immediately before beginning the 5 min holding period prior to releasing the monkeys into the field. Doses of 0.8, 4, 12, and 16 mg/kg i.m. were used in tests in both the bare open field and with novel objects present. One additional test with a dose of 24 mg/kg was done in the bare field. All monkeys were tested under all conditions except for one who was ill on the day it was to receive the 16mg/kg dose in the empty open field. In the tests of responses to novel stimuli in the open field, eight different objects were used as the novel stimuli. The objects were arbitrarily divided into sets of two. On a test day, four monkeys would be

exposed to one object in a set and the other three to the other object of that set. The next day, each monkey was exposed to the object in the set that he had not encountered before. This was continued until each monkey had been exposed to all eight objects over eight days of testing and had received 4 caffeine doses and 4 saline injections. The order in which the drug doses were given was 4, 12, .08, 16, and 24 mg/kg in the tests in the empty field and 16, 4, 12, and .08 mg/kg with the novel objects. Additional tests in the bare field with the 16 and 24 mg/kg doses were done after the tests with novel objects had been completed.

The activity scores of the animals are summarized in Table 20 which also lists the social rank of each male. Data from the tests in the bare field are given in 20a and from the novel object tests in 20b.

In the bare open field condition, locomotor activity increased significantly at one or more doses in 6 of the 7 monkeys. There were considerable individual differences in the dose response curves between animals. The exception, Tag, was one of two animals that showed considerable variation in his activity between the 4 saline days prior to the tests with the novel object and the 2 saline days after the tests with the novel object. His mean activity score (+/- S.E.M.) for the first 4 saline days was 46.8 (+/- 1.7) compared with 57.5 (+/- 9.8); thus, his activity was increased at the 0.8 mg/kg dose and the scores at the two highest doses were actually below the mean of the last 2 placebo days, which was 79.0. In all but two cases, the greatest increases in activity occurred with intermediate doses of caffeine, suggesting the presence of the U-shaped curve which the literature had led us to expect. Placing a novel object in the open field had a small, inconsistent effect on locomotor activity under placebo conditions (saline column of part a compared to part b of Table 20). The effects of caffeine were highly variable and there is no consistent pattern discernible. Perhaps there are competing response tendencies between locomotor activity and visual attention to the novel stimuli which in turn are interacting with individual differences in responsiveness to the drug.

Table 20

Locomotor Activity Under Caffeine Sodium Benzoate

a. Empty Open Field:

Animal	Rank	Saline Mean 6 Tests (+/- SEM)	Caffeine (mg/kg) 0.8	4	12	16	24
BARKER	1	19.8 (3.1)	16	23	32	46	25
EJU **	2	7.7 (3.9)	10	5	20	6	2
WEED	3	123.8(17.0)	96	168	170	-ill-	64
TAG	4	57.5 (9.8)	68	46	58	54	53
ALLEN	5	45.3 (6.6)	10	70	75	34	54
HOBBIT	T 6.5	66.8 (3.0)	58	102	88	70	91
KUKLA	T 6.5	33.0 (3.3)	30	18	20	44	75

b. Novel Object Present:

		Mean 4 Tests					
BARKER	1	19.8 (5.1)	21	17	12	23	-
EJU **	2	12.8 (3.6)	7	14	8	16	-
WEED	3	96.5 (6.2)	67	128	71	109	-
TAG	4	44.8 (9.9)	24	53	28	58	-
ALLEN	5	30.3 (2.4)	32	34	47	34	-
HOBBIT	T 6.5	71.5 (4.0)	61	66	75	64	-
KUKLA	T 6.5	35.0 (4.8)	24	26	33	24	-

** On every test but one, Eju had to be pushed into the field after the 5 min latency period.

Analysis of the individual dose response curves for emergence latencies in the bare field showed these scores to be substantially shorter at one or more doses of caffeine in 4 of the 6 animals. (Eju did not emerge voluntarily on any caffeine day and did so on only one of the six saline days.) The other 2 monkeys' latency scores were not affected by caffeine except that one exhibited a much longer latency at the 24 mg/kg dose. As compared to the first 4 days of saline injections, mean emergence latencies of all 6 monkeys were shorter and variability was much reduced on the two placebo days following the tests with novel objects. Thus, the animals entered the bare open field more quickly after being given a number of experiences. With a novel object present in the field, the emergence latencies on the saline days (mean = 3.5 sec +/- 0.3) did not differ from the latencies on the last two tests in the bare field (mean = 4.2 sec +/- 0.6) in the 6 monkeys that always emerged voluntarily. There were no consistent changes in emergence latencies in the tests with a novel object. Three of the 6 showed substantial increases in latency following one or more doses of caffeine, but the others were unaffected or had slightly shorter emergence times at one or more doses.

In tests with the novel objects, scores were obtained on the total number of interactions with the object, the number of noncontact (orienting) responses, nonaggressive contact responses (sniffing, manipulating, sitting next to), total aggressive responses (biting, threatening, etc.), and total contacts (a combination of aggressive and nonaggressive contact scores.) No fear or submissive responses were seen during these tests. Total responses to the novel object increased at one or more doses of caffeine in 6 of the 7 animals; 5 of these made the most responses to either the 12 or the 16 mg/kg dose, while the 6th peaked at 4 mg/kg. The frequency of orienting responses tended to be unchanged by caffeine - the increases were in contact and aggressive responses.

Although there was no relationship between absolute scores on any of these measures and the social status of the animals, there was a high positive correlation (rho =+.89) between frequency of agonistic behaviors in the social group and the percentage of interactions in which the animal made some sort of contact with the novel object. In addition, a greater proportion of responses to the novel object by high ranking animals involved physical contact with the object than was the case for lower ranking animals.

Effects of atropine on open field behavior. A study of the effects of atropine sulphate and atropine methyl nitrate on emergence and activity in the empty open field was completed using the seven adult males from NT-Troop. Intramuscular doses of .032, .08, and .20 mg/kg of both drugs were alternated with placebo days (physiological saline) until all animals had received all doses. There was a delay of 30 min between injection of the drug and the beginning of testing. Emergence time and locomotor activity data for each drug day were compared with the means of these measures for the 8 saline days. Monkeys failing to enter the field within 5 min of the opening of the guillotine door were gently forced into the field. This happened on two occasions during testing with the placebo but not during tests with the drugs. The data for each monkey are given at the top of Table 21. After this study had been completed, we decided to add a dose of .40 mg/kg to some of our behavioral tests. The animals were tested again in the bare open field with this higher dose after the experiment with atropine and novel objects described below was finished. These data are presented in the lower part of Table 21 and are compared with the two placebo days used with these tests.

There were no consistent effects of either drug on emergence latency at the three lower doses. There were several very long emergence latencies, particularly with atropine sulphate, but these were not well correlated with the doses given. However, at the .40 mg/kg dose of atropine sulphate (but not atropine methyl nitrate) all seven animals had longer emergence latencies. Instances of both increases and decreases in locomotor activity were observed at all doses of both drugs when compared with the means for saline days, but in most cases the scores were within the range exhibited during saline days. Thus, doses of these drugs which disrupted operant and complex problem solving behavior (see later sections of this report) had no obvious effect on locomotor activity. This was surprising in that the pilot work on activity with C-Troop had demonstrated a reduction in general activity at the .20 mg/kg dose. Finally, there was no evidence that the order of administration of the various doses across days had any effect on the data.

Table 21

Effects of Atropine Sulphate (AS) and Atropine Methyl Nitrate (AMN) on Emergence and Locomotor Exploration in the Empty Open Field

Emergence Latency (Sec):

			Drug Dose (mg/kg)					
	Placebo *		.032		.08		.20	
Animal	(+/- SEM)		AS	AMN	AS	AMN	AS	AMN
Barker	2.5	(0.34)	1	2	2	2	4	3
Weed	2.8	(0.36)	4	3	2	3	6	3
Eju *	8.1	(3.30)	5	4	5	14	9	12
Allen	13.4	(4.16)	260	6	7	13	29	6
Tag *	21.0	(10.80)	106	25	19	3	143	3
Hobbit	3.2	(0.65)	3	5	5	2	2	2
Kukla	24.2	(8.50)	259	3	9	14	132	10
Number of Moves:								
Barker	61.5	(8.92)	80	53	40	77	73	108
Weed	205.4	(8.01)	228	216	191	195	178	205
Eju *	23.3	(5.03)	24	28	33	18	26	5
Allen	46.1	(6.02)	79	25	28	29	7	39
Tag *	102.2	(6.62)	102	96	126	81	147	59
Hobbit	69.8	(6.68)	69	67	58	97	96	73
Kukla	46.8	(4.84)	32	39	20	22	50	32

*Placebo n = 8 days except 7 days emergence latency for Eju and Tag who each had a forced entry on one day.

Emergence Latency (Sec):

	Drug Dose (mg/kg)		
	Placebo	.40	
Animal	(2 day mean)	AS	AMN
Barker	1.5	4	1
Weed	1.0	4	3
Eju	3.5	100	3
Allen	3.5	105	2
Tag	2.5	38	3
Kukla	46.0	104	11
Hobbit	2.0	6	2
Number of Moves:			
Barker	43.0	36	53
Weed	236.0	232	294
Eju	15.5	6	29
Allen	51.5	48	52
Tag	118.5	111	101
Kukla	35.0	34	37
Hobbit	39.5	65	35

In a second experiment, conducted eight months after completion of the study in the bare field, a novel object was placed in the center of the field and the animals were tested with doses of .08, .20, and .40 mg/kg of both atropines. To minimize habituation, different objects were used each day. Two objects were used each day with different animals, such that some animals were exposed to a specific object on a drug day while others were exposed to the same object on a placebo day. Objects were either large toys, such as a hobby horse, or household items such as a ladder, a vacuum, a bucket, etc. Drug and placebo days were alternated until all monkeys had received all three doses of both drugs. The results are given in Table 22.

Table 22

Effects of Atropine Sulphate (AS) and Atropine Methyl Nitrate (AMN) on Behavior in the Open Field Containing a Novel Object

Emergence Latency (Sec)

		Drug Dose(mg/kg)					
	Placebo *	.08		.20		.40	
Animal	(+/- SEM)	AS	AMN	AS	AMN	AS	AMN
Barker	1.3 (0.25)	1	2	2	4	5	3
Weed	2.3 (0.63)	1	2	2	4	3	2
Eju *	2.7 (0.29	2	3	18	6	88	3
Allen	4.8 (1.38)	2	2	12	4	10	3
Tag	2.3 (0.48)	2	2	3	2	2	2
Kukla	17.0 (2.68)	4	5	13	9	3	1
Hobbit	3.5 (0.87)	3	6	5	3	9	1

Number of Moves

Barker	48.8 (3.83)	41	38	67	52	6	36
Weed	198.8 (12.43)	189	240	192	218	137	279
Eju *	31.8 (6.82)	32	20	45	51	17	25
Allen	83.0 (8.53)	78	79	57	108	6	34
Tag	81.8 (11.99)	104	63	73	102	68	44
Kukla	56.5 (4.30)	69	50	33	44	33	39
Hobbit	42.5 (5.01)	54	18	82	80	31	47

* Placebo n = 4 days except 3 days for Eju who had an emergence latency of 25 sec on one day.

In tests with a novel object present in the open field, the .08 mg/kg dose of either atropine sulphate or atropine methyl nitrate had little effect on emergence latencies in 6 of the 7 animals. (Kukla, however, had a much shorter latency.) If anything, the animals entered the field a little bit faster with this dose of atropine sulphate. With the two higher doses of atropine sulphate, but not atropine methyl nitrate, 4 of 7 monkeys showed a dose dependent increase in emergence latencies similar to that seen with the .40 mg/kg dose in all 7 animals in the bare field study; 2 exhibited no effect, and 1 a much shorter latency when compared to placebo scores.

The .40 mg/kg dose of atropine sulphate produced a decrease in locomotor exploratory behavior in all seven monkeys. A few animals also had a decrease at .20 mg/kg, while no differences appeared with .08 mg/kg. Four animals had reduced activity scores with the .40 mg/kg dose of atropine methyl nitrate, 2 were unchanged, and 1 exhibited an increase. In the 4 animals with the lower scores, the reductions were smaller with atropine methyl nitrate than those obtained with atropine sulphate. The results with the.40 dose are the first consistent differences between atropine sulphate and atropine methyl nitrate effects which we had seen on any of our tests with these drugs. The differences appear only with a relatively large dose. The overall picture suggests that there is an interaction between the central and peripheral effects of the two drugs on open field behavior. Perhaps pretreating the animals with atropine methyl nitrate and then give varying doses of atropine sulphate would provide an indirect assessment of central effects on this task.

Correlations between open field and social variables. In the caffeine study (above), we had found a high positive correlation between the monkeys' agonistic behavior frequencies in the group and the percentage of their responses to the novel objects that were contact responses. However, this relationship was not present on placebo days in the atropine study. There had been changes in social rank prior to the atropine study and these changes were not accompanied by the corresponding changes in percent contact that would have been predicted from the earlier correlation. In addition, low ranking monkeys showed an overall increase in contact responses relative to their performance the first time around. It appears that there was habituation to the general test situation such that lower ranking animals were now more willing to approach and contact the objects.

There were high correlations between short emergence latencies and high social rank in the NT-Troop males on placebo days during both the empty field (+.79)and the novel object

present (+.78) phases of the atropine experiment. We had not seen this before in NT-Troop, but the earlier data contained many instances of nonemergence in these males.

Effects of diazepam on open field behavior. The effects of diazepam on emergence and activity were studied in the open field with a novel object present. Unlike the experiments with caffeine and atropine, no testing was done with the empty open field. The subjects were once again the NT-Troop males, but one animal, Tag had died during the preceding year and he was replaced by Ouzel, a six year old monkey that had not been tested previously.

Animals were given i.m. injections of either drug or placebo (diazepam vehicle) 15 before testing was to begin. Ten minutes later they were taken to the apparatus and testing was started after the usual 5 min delay. Ten tests were run over a period of 12 days; there were 4 diazepam days and 6 placebo days. The first two days were placebo days as were the 4th, 8th, 10th, and 12th days. No testing was done on the 6th and 7th days. Diazepam doses were 0.16, 0.80 (twice), and 1.60 mg/kg. The order of the doses was 0.80, 1.60, 0.16 and 0.80. Two observers were used and the means of their scores were used in the analyses of the data.

Ouezel, the new animal, did not meet the 5 min criterion for voluntary emergence on either of the first two placebo days. He was allowed extra time and did enter the open field with latencies of 345 and 500 seconds. We continued to test him for the remainder of the experiment but, although he met the emergence criterion on subsequent days, his data are not included in the analyses which follow. The object placed in the open field on day one (placebo), which was considered to be a warmup day for both observers and animals, was a large pink plastic baseball bat, mounted vertically. The animals had been exposed to the bat in an earlier experiment. On the second day (placebo) and third day (first .80 mg/kg dose of diazepam) rubber fright masks which the monkeys had never seen before were stuffed and mounted on top of the bat. The animal's responses to the masks were extreme. The two lowest ranking animals refused to enter the arena voluntarily on either day and had to be gently forced in before the tests could continue. The mean emergence latencies on these two days are compared with the day 1 and day 12 placebo and the day 11 (0.80 mg/kg diazepam) latencies in table 23. The objects on days 11 and 12 were a stepladder and a blue handtruck.

Table 23

Mean Open Field Emergence Latencies (sec) on Placebo (3 days) and 0.80 mg/kg Diazepam (2 days) with Different Stimulus Objects (n=6 monkeys)

Test Day	1	2	3	11	12
Drug	Placebo	Placebo	Diazepam	Diazepam	Placebo
Objects	Bat	Mask A or B	Mask B or A	Truck or Ladder	Ladder or Truck
Emergence Latency	10.9	122.2	118.3	25.0	6.9
(+/- SEM)	(4.3)	(62.9)	(63.3)	(11.7)	(2.4)

Because of the variability in the data, a square root transformation was performed ($x_t = [x+0.5]^{1/2}$) and a one-way within subjects ANOVA performed. A significant result ($F_{4,20} = 5.32$, $p < .01$) led to a Newman-Keuls test of the individual means which yielded significant differences between both mask days and the other three days. The diazepam "truck or ladder" day (day 11) was not different from placebo days 1 or 12 in these comparisons. Because the masks produced such a marked change on emergence latencies on days 2 and 3, the data for these two days were examined separately from those of the last seven days of the experiment. On the measure of locomotor activity, mean number of moves was 58.2 (+/- 21.5) on the placebo day and 50.2 (+/- 21.6) on the diazepam day. (The mean on day 1 had been 75.3 (+/- 25.7.) Two monkeys incresed and four decreased their scores on day 3. However, there was a consistent decrease in the number of contact responses the monkeys made with the masks on the diazepam day (means: placebo = 5.5, diazepam = 2.8; $t = 2.65$, df 5, $p < .05$).

The remainder of the experiment was conducted with "neutral" novel objects such as the ladder and handtruck mentioned above. The emergence latency and locomotor activity data are given in table 24.

Table 24

Mean Open Field Emergence Latencies (sec) and Locomotor Activity Scores (Moves) for Three Diazepam (DZ-mg/kg) and Four Placebo (PL) Days with Stimulus Objects Present (n=6).

Test Day	4	5	8	9	10	11	12
Drug	PL	DZ-1.60	PL	DZ-0.16	PL	DZ-0.80	PL
Emerg. Lat.	25.9	20.3	14.9	11.6	10.8	25.0	6.9
(+/- SEM)	(14.2)	(4.4)	(5.2)	(4.5)	(5.7)	(11.7)	(2.4)
Moves	88.3	52.5	76.8	59.5	81.1	34.5	59.3
(+/- SEM)	(27.9)	(15.2)	(24.5)	(19.9)	(23.3)	(11.7)	(28.9)

A one-way within subjects ANOVA of the transformed emergence latency data using the mean of the four placebo days as the control produced a significant drug effect ($F_{3,15} = 3.57$, $p < .05$. However, post hoc Tukey tests revealed only marginal differences between 0.80 and 1.60 mg/kg and the 0.16 mg/kg and placebo groups. As there was a statistically significant decrease in emergence latencies across the last three placebo days, each drug dose was compared with the mean of the placebo day immediately preceding and following that drug day. Once again, the drug effect was significant ($F_{1,5} = 14.6$, $p < .02$) but the post hoc tests reached significance only on the comparisons between the 0.16 mg/kg group and the two higer doses of diazepam. We concluded that diazepam produces a small increase in emergence latency.

Locomotor activity was reduced at the two higher doses of diazepam. Since the number of moves scored across the last four placebo days did not change significantly, the locomotor activity data were also analyzed by using the mean of the placebo days in the one-way ANOVA. There was a significant drug effect ($F_{3,12} = 5.44$, $p < .01$). Post hoc tests showed the difference between the .080 mg/kg group and the placebo and 0.16 mg/kg groups to be significant. The difference between the .080 and 1.60 mg/kg groups was not significant and the apparent U-shape of the dose response curve probably can be discounted. We looked at the patterns of the scores for the placebo days immediately preceding and following each drug day and found that the slightly higher mean in the 1.60 mg/kg group (see Table 24) was due to one animal which had a slight increase in activity on this day.

There was no overall effect of diazepam on the frequency of responses directed toward the stimulus objects during the last seven days of the experiment. Although the data suggested that there might be a small, dose dependent increase in contact responses, the overall $F_{3,15} = 1.856$ was not significant. An examination of the pattern of responding by individual monkeys showed that there was a consistent increase in contact responses at the 1.60 mg/kg dose when compared to the mean of the placebo days immediately preceding and following the drug day. ($t = 2.57$, df 5, $p < .05$). At both the 0.80 and 1.60 mg/kg doses, there was a tendency for the animals with the lowest social rank to show the largest increase in frequency of contacts.

E. Complex Problem Solving:

The six oldest adult males in T-Troop were used in the studies on complex problem solving. Three of the monkeys had previous experience on the task and three were experimentally naive. Performance was related to social behavior and status and the effects of caffeine, the atropines, and diazepam on performance were investigated. Because previous work (Bunnell and Perkins, 1980b) had shown a relationship between social variables and performance on this task, it was thought to have considerable potential for inclusion in the test battery. It requires a very labor intensive procedure, however, so particular attention was paid to the course of training on the problems, and to the ease with which the animals could be retrained following breaks in testing caused by schedule requirements.

The problem solving situation required the animals to acquire a "win-stay lose-shift" strategy to be successful. On this task, which was conducted in a modified Wisconsin General Test Apparatus (WGTA), the monkeys were trained on a series of 10-trial object quality learning set problems until they reached a criterion of 17 correct trial two responses in 20 consecutive problems. Each problem required the animal to learn which of two objects had a raisin underneath it. No matter what the performance on a problem, a new problem, with a new pair of objects, was presented after 10 trials on the original problem. A large number of plastic, metal, and wooden toys and hardware items of differing colors, shapes and sizes were used as the stimulus objects. Over many different problems the animals acquire a learning set - that is, each time they are faced with a new pair of objects, the first response provides the solution to that problem. If the animal picks up the object and finds a raisin, it continues to respond to that object for the rest of the problem; if there is no raisin under the object selected on the first trial, it chooses the other object on the second trial. Thus the first trial on each problem provides the cue as to which object is correct and performance on the second trial is the key for demonstrating that the win-stay lose-shift strategy has been learned.

After criterion was reached on the object quality learning set problems, training on a reversal learning set was initiated. In this condition, the animals were given four new problems each day, with lengths of 10, 11, 12 and 13 trials. (The order of presentation of problems of different length was counterbalanced across days). Reversals occured on the fifth trial of the 10-trial problems, the sixth trial of the 11-trial problems, etc. When a reversal took place, the object that had been correct up to that trial of the problem was no longer rewarded and the other object of the pair became the correct

stimulus for the remaining five trials on that problem. Criterion performance was 17 out of 20 correct critical trial responses in 20 consecutive problems. The critical trial on a problem was the first trial after the reversal trial which provided the cue for the monkey to shift its response to the other object. The intertrial interval was 30 sec and the monkey was allowed a maximum of 10 sec to respond to each stimulus presentation. There were a total of 46 trials per daily session and each session was 25-30 min long.

Measures of learning and performance obtained on this task were: Habit Formation - the intraproblem performance on each new problem up until the reversal trial was given, defined as the number of correct responses on initial learning of each day's four problems. Concept Formation - assessed on both the object quality learning set and the reversal learning set portions of the problems. As indicated above, correct responses on the second trial of each new problem across successive problems provided the measure of object quality learning set performance and correct responses on the critical trials across problems were the measure of reversal learning set performance. In addition, total errors, anticipatory reversal errors, and response patterns, e.g. perseveration of responding to particular positions or objects, the development of response strategies, and the like, were also examined. To provide flexibility in the testing program, a minimum of two trained assistants were always available to conduct the tests so that the monkeys were adapted to being tested by different experimenters. Additional details of the training and testing procedures may be found in Bunnell and Perkins (1980b). A description of the WGTA apparatus is given in Bunnell, Gore and Perkins (1980a).

Retraining of the experienced animals took five months. At this time the three inexperienced animals all were having days on which they had three or four errorless reversals on the four daily problems; it was seven months before they reached criterion performance. During the course of the project, testing was interrupted for periods of time of from one week to six months. Interruptions of as long as a month produced virtually no performance decrement, while criterion performance could be restored in 2-3 weeks after 3-4 months without testing. In one instance, following a six month interruption, animals were tested every other day and it took about five weeks for four of the monkeys to reach criterion. The other two animals had many response failures, and although it was clear that they had not forgotten the task, it required additional daily testing to produce criterion performance.

In our earlier work with this task, we had employed a procedure whereby the animals were required to ignore the reversal cue once criterion had been reached on the reversal

learning set problems (Bunnell and Perkins, 1980b). The reversal learning set was extinguished by reversing the correct stimulus for only one trial (false reversal trial) after which the originally correct stimulus was again reinforced for the remainder of the trials on that problem. During this extinction procedure, the animals made many more critical trial errors than they did during acquisition, and it was several months before the criterion of 17 out of 20 correct responses on the trial following the false reversal trial was met. Subsequent retraining on the reversal task took as long as initial acquisition. The length of time involved in acquisition-extinction-acquisition, etc. made inclusion of such a procedure in the test battery impractical. However, the difficulty the animals had in extinguishing the reversal set suggested that a modified extinction procedure might be useful in assessing drug effects. Once the monkeys had reached criterion on the reversal task, their performance tended to be quite stable across days. This provided the opportunity to evaluate drug induced performance decrements, but left very little room to assess drug effects that might enhance performance or prevent decrements. We conducted an experiment to assess the effect of introducing an occasional extinction problem among the reversal problems. Following three baseline days of regular 4-problem reversal tests, "false reversal" problems were introduced randomly over the seven days. The study concluded with two more baseline reversal days. During the seven days different monkeys received anywhere from 3 to 6 false reversal problems, as not all animals got a false reversal problem each day. The data following each false reversal were examined for effects on the remaining trials on that problem, carryover to subsequent regular reversal problems on the same day, and carryover to following days.

The results were disappointing. Each animal responded to the reversal manipulations in an idiosyncratic manner and there were marked differences in both the degree and kind of disruption of performance produced by the false reversal cue. Three animals learned to ignore the false reversal cue. That is, they would reverse on cue by responding to the reversed stimulus on the next trial, find no reinforcer, and switch back to the originally correct stimulus for the remaining trials of the problem. One of the monkeys began doing this on the second exposure to the false reversal cue and exhibited no carryover effect on subsequent reversals, i.e., if the reversal cue was true insted of false, no errors occurred for the remainder of the problem. A second animal also ignored the false reversal cue after one exposure, but this monkey then failed to respond correctly to true reversal cues on one or more subsequent problems on that day. It also exhibited some response failures and there was a carryover to the next day following one of the false reversal problem days. The third monkey continued to respond to the false reversal cue as if it were true for five

consecutive days. However, on subsequent problems given that day, it was impaired on the true reversal problems and did not respond consistently to the reversed stimulus. On the sixth and seventh days it ignored the false reversal cue and showed no impairment on subsequent true reversals. Of the remaining three animals, one responded to the reversal cue every day and showed no carryover effect on subsequent problems; however, it had many response failures and these carried over to subsequent days. Another responded to the false reversal trial on the first day, ignored it on the second, and then went back to reversing on cue on the third day during which it was impaired on subsequent reversals. It continued to respond to the false reversal cue as if it were true on its last two exposures, but there was no effect of subsequent problems. The sixth animal's pattern was similar except that it ignored the false reversal cue on the first two exposures. Thus, although the false reversal procedure produced performance deficits, the way in which individual monkeys dealt with the situation was so variable as to cast doubt as to its utility for producing the kind of reliable behavior changes that would be needed for generating dose response curves or studying chronic drug effects.

Relationships Between Social Behavior and WGTA:

In an earlier study of the relationships between social status and WGTA performance (Bunnell and Perkins, 1980b) we found that high ranking males made more errors on critical trials during reversal learning set training than did low ranking males and took longer to extinguish the reversal set. In retraining the three oldest monkeys that had previous experience on the task, the same relationship was observed; however, the relative ranks of these animals were the same as they had been in the initial study, so the significance of this finding is questionable. Nevertheless, the relationship appeared again among the three inexperienced animals during their training on reversals - Yaztremsky, ranked sixth among the males, reached criterion first, followed by Sky, ranked fifth, and, finally, Vulcan, the fourth ranked animal. There was a spontaneous change in rank in the troop in September, 1984; following the first administration of the .40 mg/kg dose of atropine sulphate, Easy, the top ranked monkey, was replaced by Oliver, who had ranked third. Madison dropped to third and the ranks of Vulcan, Sky, and Yaztremsky stayed the same. The resolution of this change in the male dominance hierarchy was completed over the next week to 10 days. During the saline days in this time period, there was no change in either Oliver's or Easy's scores that might be considered to reflect the altered social structure of the troop. Apparently, once performance has stabilized, the relationships between high social rank and poor performance disappear or change (see the next paragraph and Table 25). In addition, there was no obvious correlation

between changes in performance produced by the false reversal cues (see above) and any of the social variables.

Nevertheless, some interesting relationships between social variables and performance were present during the time performance was stable at criterion levels. The correlations among social rank, frequency of submissive, aggressive, and "other social" behaviors, mean daily Trial 2 Correct responses, mean daily Critical Trial Correct responses, and mean total reinforced responses on all 46 daily trials (an indicant of overall daily performance in the WGTA) are given in Table 25. The data cover 20 days of social observations during May, 1985 for which 18 days of WGTA data were also obtained. Although the small number of animals requires that the correlations be interpreted with caution, some interesting relationships are apparent. Trial 2 Correct responses on initial learning and total reinforcements received are negatively correlated with frequency of submissive behaviors and positively correlated with high social rank. Thus, monkeys that make few submissive responses do well on the object quality learning set part of the task and make more correct responses overall each day when they are performing at criterion levels over an extended period of time. As noted above, this is quite different from the relationships obtained in the earlier study involving acquisition and extinction where high ranking animals were slower reaching criterion performance and took longer to extinguish the reversal set.

Although both Trial 2 Correct and Critical Trial Correct scores are positively correlated with overall performance as measured by total reinforcements received, their intercorrelation is a nonsignificant +.41. This indicates that the two parts of the task are tapping different dimensions of the monkeys' performance in the complex problem solving situation.

Drug Effects on WGTA Performance:

Effects of caffeine on WGTA performance. In the first experiment, i.m. doses of 12, 4, and 0.8 mg/kg caffeine sodium benzoate were administered im 5 min before testing was begun. (The rationale for the selection of these doses as the initial doses is given in the appendix, which contains the caffeine protocol). Drug days alternated with placebo days (physiological saline) until all animals had received each dose of the drug. (The order of the doses was 12, 0.8, & 4 mg/kg.) Animals were tested 5 days a week, with Monday always being a placebo day to account for warmup effects. Drug and placebo solutions were coded so that neither the persons administering the injections nor the experimenters doing the testing knew what the animals were getting. There was a delay of 5 min between injection of the drug and the start of testing. The data are presented in Table 26.

Table 25

Correlations Between Social Variables and Performance on the WGTA

	NUMBER SUBMIS-SIVE	NUMBER AGGRES-SIVE	NUMBER OTHER SOCIAL	TRIAL 2 CORRECT	CRITICAL TRIAL CORRECT	TOTAL REIN-FORCERS
SOCIAL RANK	-.94 *	.83 *	.20	.81	.54	.89 *
NUMBER SUBMIS-SIVE		-.60	-.37	-.99 *	-.37	-.83 *
NUMBER AGGRES-SIVE			.14	.56	.09	.54
NUMBER OTHER SOCIAL				.50	.14	.31
TRIAL 2 CORRECT					.41	.84 *
CRITICAL TRIAL CORRECT						.83 *

* $p<.05$

Table 26

Effects of Caffeine Sodium Benzoate on WGTA Performance: Experiment 1
[Means (+/- SEM) for Habit Errors, Total Errors, Object Quality Learning Set (Trial 2 correct out of 4) and Reversal Learning Set (Critical Trial correct out of 4)]

	Dose (mg/kg)			
	Saline (3 day Mean)	0.8	4.0	12.0
Habit Errors	3.40 +/- (.63)	5.17 (1.25)	3.67 (.88)	4.50 (.47)
Total Errors	7.55 +/-(1.01)	8.67 (1.35)	7.17 (1.43)	9.17 (1.34)
Trial 2 Correct	3.40 +/- (.32)	3.17 (.34)	3.83 (.18)	3.17 (.34)
Critical Trial Correct	3.10 +/- (.07)	2.83 (.34)	2.83 (.66)	2.17 (.77)

There were no significant effects on performance by the three doses of caffeine. However the animals did make slightly more habit errors at the 0.8 and 12 mg/kg doses and 6 out of 6 monkeys made more total errors at the 12 mg/kg dose. They did slightly better on the object quality learning sets at the 4 mg/kg dose and slightly worse on reversal sets at all three doses when compared with the means of the saline days. Of particular interest were the tendencies suggesting that object quality learning set performance might be better at 4 mg/kg, while reversal set performance seemed to be deteriorating, particularly at the 12 mg/kg dose, in some of the monkeys. (The increased interanimal variability reflects the fact that some animals continued to perform at criterion levels while others were severely impaired. It appeared that individual differences in dose response curves might be obscuring a drug effect.) Accordingly, another experiment was done in which the 4 mg/kg dose was repeated (twice) and doses greater than 12 mg/kg were added to see if reversal performance would be poorer in all of the subjects at one or more higher doses.

The second experiment used doses of 24, 16, 8, 4 (twice) and 2 mg/kg with the order of administration being 16, 4, 2, 8, 4, & 24. The testing schedule was arranged so that 72-96 hours elapsed between the higher doses (8, 16, and 24 mg/kg) and the next administration of caffeine. The usual double blind procedure was followed and there was a 5 min delay between drug administration and testing. Performance on drug days was compared to average performance across seven placebo days

(Mondays were excluded from this baseline.)

When the data were examined, it was found that five of the six monkeys exhibited a marked reduction in habit errors with the 8.0 mg/kg dose, and only the 8.0 mg/kg dose, of caffeine. the sixth monkey showed the same error reduction and a similar pattern of responding on the problems with the next highest dose, 16 mg/kg. In analyzing the data, we realigned this animal's scores at 16 mg/kg with the 8 mg/kg scores of the other five animals (this is the rough equivalent of matching their ED 50 scores) on all four dependent variables. The data, which include this realignment, are given in Table 27.

Table 27

Effects of Caffeine Sodium Benzoate on WGTA Performance: Experiment 2 [Means (+/- SEM) of Habit errors, Total Errors, Trial 2 Correct and Critical Trial Correct]

	Dose mg/kg					
	Saline (7 days)	2.0	4.0 (2 days)	8.0	16.0	24.0
Habit Errors	4.77	3.50	4.25	1.00	4.50	5.08
+/-	(1.03)	(.73)	(1.09)	(.81)	(1.15)	(.63)
Total Errors	8.23	5.83	6.75	5.00	6.83	7.67
+/-	(1.20)	(.96)	(1.63)	(1.55)	(1.71)	(.97)
Trial 2 Correct	3.12	3.00	3.25	3.83	3.17	3.67
+/-	(.17)	(.40)	(.39)	(.18)	(.46)	(.23)
Critical Trial Correct	3.17	2.83	3.33	3.00	2.83	3.00
+/-	(.14)	(.44)	(.14)	(.49)	(.38)	(.40)

A one-way within subjects analysis of variance of the habit formation error data yielded a $F_{5,25} = 5.39$, $p < .01$. Post hoc Tukey tests showed that the error scores for the 8 mg/kg dose were than lower than those of the placebo and all of the other caffeine doses except the 2 mg/kg dose. The lower habit error scores are reflected in the better Trial 2 performance at the 8 mg/kg dose, but there is a ceiling effect operating and the improvement is not significant.

A third experiment, conducted in conjunction with the first atropine experiment described in the next section, included a dose of 36 mg/kg of caffeine and another 4 mg/kg dose. With the 36 mg/kg dose, one animal stopped responding halfway through the session. The data for the five monkeys that responded on all problems are given in Table 28.

Table 28

Effects of Caffeine on WGTA Performance: Experiment 3 (n = 5)
(Means +/- SEM)

	Dose mg/kg		
	Saline (4 days)	4.0	36.0
Habit Errors	3.22 (0.72)	4.20 (1.32)	1.40 (0.68)
Total Errors	6.88 (1.26)	7.60 (1.17)	5.80 (1.80)
Trial 2 Correct	3.28 (0.18)	3.00 (0.32)	4.00 (0.00)
Critical Trial Correct	2.88 (0.25)	2.80 (0.20)	2.60 (0.51)

With the 36 mg/kg dose, there was a nonsignificant drop in habit formation errors ($F_{2,8} = 2.13$, $p = .18$). Since all 5 monkeys had perfect scores on their object quality learning sets, the appearance of a significant F ($F_{2,8} = 7.50$, $p < .02$ was not surprising; however, the Tukey tests showed that 36 mg/kg scores differed only from the 4 mg/kg scores and not the placebo scores.

The 4 mg/kg dose had been selected originally because it produced an increase in locomotor activity whereas the 12 mg/kg dose had depressed locomotion (see the section on activity and drug dose selection). The trend in the data from the first experiment was toward slight improvement at 4 mg/kg and slight impairment at 12 mg/kg. Therefore the 4 mg/kg was repeated in experiments 2 and 3 along with a dose intermediate between 4 and 12 mg/kg (8 mg/kg) and higher doses (16, 24, and 36 mg/kg). The 8 mg/kg dose did reduce error scores but the higher doses did not produce the deterioration of performance we had anticipated as a result of the 12 mg/kg data - in fact, object quality learning set (Trial 2 scores) actually improved in 5 of the 6 monkeys at the 24 and 36 mg/kg doses. The 4 mg/kg dose was given in all three experiments. For comparison purposes, data for the four administrations of this dose are presented in Table 29. There were no significant drug effects and performance under placebo conditions was fairly stable over the three months of testing.

Table 29

Four Successive Administrations of 4mg/kg Caffeine Sodium Benzoate and WGTA Performance

	Experiment 1		Experiment 2			Experiment 3	
	saline 3 days	drug	drug	saline 7 days	drug	saline 3 days	drug
Habit Errors	3.40 (.63)	3.67 (.88)	4.73 (1.71)	5.20 (.46)	3.50 (1.01)	3.40 (.67)	4.00 (1.20)
Total Errors	7.55 (1.01)	7.17 (1.43)	8.40 (2.82)	8.23 (1.20)	5.33 (1.00)	6.90 (1.12)	6.50 (1.26)
Trial 2 Correct	3.40 (.32)	3.83 (.18)	2.80 (.80)	3.12 (.17)	3.67 (.23)	3.32 (.16)	3.16 (.34)
Critical Trial Correct	3.10 (.07)	2.83 (.66)	3.20 (.58)	3.17 (.14)	3.50 (.24)	2.90 (.22)	3.00 (.28)

The caffeine data from the complex problem solving task, when viewed across the three experiments, indicate that caffeine had only small and transient effects on performance. Some improvement was seen first at the 8 mg/kg dose and appeared later at doses of 24 and 36 mg/kg. Some behavioral tolerance appeared to have developed, but this possibility was not examined further. In a few instances, some monkeys were slightly impaired, as reflected in the increased variability of the group means - generally these were the animals that performed at lower levels on placebo days. There was no dose which impaired performance of all of the animals on any of the measures taken. There was nothing to suggest that associative processes were affected by the drug or that changes in overt activity levels influenced responding. What drug effects that did appear were probaly attributable to attentional factors; however, the results did not seem very important and we did not pursue the matter further.

With regard to the evaluation of the utility of the task in the test battery, the caffeine studies do provide some useful information. First, once criterion performance is achieved, behavior is relatively stable across time and provides a good baseline against which to assess the effects of repeated administrations of a drug. However, since caffeine effects were small, it is not known whether or not drugs which might produce serious disruptions of performance would cause significant changes on subsequent baseline data. The one animal that stopped responding when given 36 mg/kg caffeine (experiment 3) was impaired on the placebo day which followed. This problem will be examined more thoroughly in the atropine

studies described in the next section. As noted earlier, we were concerned about the operation of a ceiling effect which would make it difficult to detect performance enhancing effects of drugs. This was a problem with the Trial 2 performance scores, but the number of habit formation errors was high enough under the baseline condition to allow the detection of error reduction. Finally, the caffeine data strengthen the idea that reversal performance may be partially independent of object quality set performance. Reversal scores also tended to be slightly lower than trial two scores across the board.

Effects of atropine sulphate on WGTA performance. One experiment was conducted on the effects of i.m. doses of .20 and .40 (given twice) mg/kg atropine sulphate on the learning set task using essentially the same procedures as employed in the caffeine studies. Dosages in this case were selected on the basis of the pilot studies cited earlier in the section on activity. Dose order was .40, .20, and .40 interspersed with placebo and caffeine trials; atropine trials were separated by a placebo day, a caffeine day, and another placebo day. A waiting period of 15 min between injection and the beginning of testing was used.

There was a dose dependent disruption of performance. The data are summarized in Table 30. Performance under .40 mg/kg is given as the mean of the two administrations of this dose. Because there was a considerable increase in response failure, errors are given as percent of the total number of responses actually made (exclusive of first trials and reversal trials on each problem).

Table 30

Effects of Atropine Sulphate on WGTA Performance
Means (+/- S.E.M.)

	Dose (mg/kg)		
Response Measure	SALINE	.20	.40
% Habit Errors	16 (3.00)	28 (4.00)	29 (5.00)
% Total Errors	19 (3.00)	39 (6.00)	51 (7.00)
# No Response	0.9 (0.70)	11.5 (4.49)	18.5 (4.62)
Trial 2 Correct	3.2 (0.16)	2.5 (0.50)	2.3 (0.38)
Critical Trial Correct	2.9 (0.12)	2.2 (0.60)	1.1 (0.29)

There were significant increases in habit errors, ($F_{2,10}$ = 4.32, p< .05) total errors ($F_{2,10}$ = 11.12, p < .01) and failures to respond at both doses. Object quality learning set performance, as measured by Trial 2 errors, appeared to be moderately impaired at both the .20 and .40 mg/kg doses, but there was considerable variability in individual performance and the differences were not significant. Significant effects were found on reversals ($F_{2,10}$ = 6.67, p < .02), but only at the .40 dose was there consistent impairment across animals on the reversal problems. In many instances, animals responded correctly on trial 2 and/or the critical trial, even though errors increased substantially on other trials of a problem. In four of the animals, errors began to appear immediately; in the other two, they increased gradually across problems on a given day. Failures to respond increased later in the day's test for most of the animals that did not respond on all trials. Failures to respond generally occurred first on the reversal phase of the problems and began to affect performance on prereversal trials on later problems. A few monkeys would occasionally refuse to take the raisin reward after making a correct choice and this tended to happen toward the end of the day's problems. The fact that this was comparatively rare suggests that the initial performance decrement was not motivational - this was supported by the activity cage study in which the animals accepted fruit readily 30 - 90 min after similar doses of atropine. When the two administrations of the .40 dose were compared - these were separated by 16 days during which there were several saline and caffeine days as well as the .20 atropine day - there was evidence of sensitization. Mean frequency of failure to respond went from 10.5 +/- 5.18 to 28.0 +/- 5.96; mean habit errors increased from 22% +/- 06% to 38% +/- 05%, and mean total errors from 37% +/- 04% to 65% +/- 01%.

In the second study, i.m. doses of .20, .08 and .032 mg/kg of both atropine sulphate (AS)and atropine methyl nitrate (AMN)were given to the monkeys. Three monkeys received the .20 mg/kg dose of AS and 3 got the same dose of AMN. All 6 animals were given the .08 and .032 mg/kg doses of both drugs.The schedule was arranged such that 3 monkeys received AS and 3 AMN on a given day. Drug days alternated with placebo (physiological saline) days, except that there was a 72 hr delay between the .20 mg/kg dose and the next test with saline. The initial study had used a 15 min delay between drug administration and testing. Because performance tended to be worse at the end of each session than it was at the beginning, this interval was increased to 30 min. The results are presented in Table 31. Since there were a number of response failures during the tests, errors were calculated as percentages of the total responses actually performed.

Table 31

Effects of Atropine Sulphate (AS) and Atropine Methyl Nitrate (AMN) on WGS 3 Performance
Means (+/- SEM)

Response Measure	Dose (mg/kg) .20 AS	.20 AMN	.08 AS	.08 AMN	.032 AS	.032 AMN	SALINE
n =	2*	3	6	6	6	5*	6
% Habit Errors	32 (02)	23 (05)	21 (05)	18 (06)	14 (04)	15 (03)	18 (04)
% Total Errors	28 (05)	27 (02)	21 (05)	20 (03)	16 (03)	16 (02)	17 (03)
# No Responses	29.0 (5.0)	2.3 (1.2)	9.5 (4.7)	20.8 (7.6)	0.7 (0.5)	0.0 -	1.0 (0.6)
Trial 2 Correct	2.0 (0.0)	2.3 (0.3)	2.2 (0.5)	2.2 (0.8)	3.3 (0.2)	2.6 (0.5)	3.6 (0.2)
Critical Trial Correct	0.5 (0.5)	3.0 (0.0)	2.0 (0.7)	1.5 (0.6)	2.8 (0.3)	3.0 (0.6)	3.8 (0.2)

*** Saline scores are based on means for 5 placebo days. Trial 2 and Reversals scores are number correct out of 4 per day. One monkey responded on only 1 trial at the .20 mg/kg dose of AS and on 10 trials at the .032 dose of AMN and these data are not included in the table.

A dose dependent impairment of performance was produced by both atropines. The 3 monkeys that received the .20 mg/kg dose of atropine sulphate performed somewhat worse than the 3 that got the same dose of atropine methyl nitrate. These differences were due primarily to the large number of response failures in the atropine sulphate group. One animal made only one response and his data are not included in the table. Examination of the data from the experiment that used a 15 min injection-test delay and included a .20 mg/kg dose of atropine sulphate suggests that the apparent differences may be a function of the particular individuals that got the atropine sulphate in the present experiment. It would be necessary to repeat this dose of both drugs with all 6 monkeys to resolve the issue. Certainly, the response failures were no greater with atropine sulphate than with atropine methyl nitrate at the .08 mg/kg dose where all 6 animals received both drugs. With the .08 mg/kg dose, the slight increase in errors over saline days is

nonsignificant, but the impairment on both learning set and reversal performance is real as is the increase in response failures. Here there is no difference between the two forms of atropine. Some, but not all of the deficits in learninn set and reversal performance are attributable to response failures since animals that continued to respond made fewer correct choices on the criterion trials for these measures. At the .032 mg/kg dose, the effects have largely disappeared although reversal performance is down slightly in the atropine sulphate group.

There is a potentially interesting relationship between the social rank of the monkeys and their performance under atropine. Using a combination of Trial 2 and Reversal Trial scores as an index of overall performance, the rank order correlation between high social rank and performance on placebo days is a nonsignificant +.61; with the .032 mg/kg dose of the atropine (combined) it is only +.20; but at .08 mg/kg, it is +.89 which yields a $p < .05$, two-tailed, despite the small n involved. At .20 mg/kg, the correlation is only +.76, but this compares closely with a +.74 obtained from data from the earlier study where all 6 monkeys received .20 mg/kg of atropine sulphate. This indicates that, at least at the moderate .08 mg/kg doses, the drug effects interact with social status such that high status monkeys show less impairment of performance than lower ranking animals. Further examination of the data for the .08 mg/kg dose yielded a correlation of -.99 between social rank and number of response failures - the higher the animals' rank, the fewer the trials on which he failed to respond. To test the robustnesss of this finding, it would be necessary to manipulate the social status of the individual monkeys and see if the apparent drug-induced interaction between status and deterioration of performance still obtained.

Effects of Diazepam on WGTA performance. The effects of diazepam doses of 0.16, 0.40, 0.80, and 1.60 mg/kg on WGTA performance were examined. There was a 15 min delay between injection of either drug or placebo and the beginning of each day's test. The experiment lasted 17 calendar days during which the monkeys were tested on 13 days (no tests were conducted on weekends) and the schedule was arranged so that there was a minimum of 72 hours between diazepam injections. The order of testing and drug administration was:

DAY:	1&2	3	4	5	6&7	8	9
	No Drug Control	Placebo	0.80 mg/kg diaz.	Placebo	NO TEST	Placebo	0.16 mg/kg diaz.
DAY:	10&11	12	13&14	15	16	17	
	Placebo	1.60 mg/kg diaz.	NO TEST	Placebo	0.40 mg/kg diaz.	Placebo	

The data are given in Table 32. There were relatively few response failures so error scores are given as frequencies instead of percent of total responses.

Table 32
Effects of Diazepam on WGTA Performance (Means +/- SEM)

	Habit Errors (out of 22)	Total Errors (out of 38)	Trial Two Correct	Critical Trial Correct
NO DRUG CONTROL	2.00 (0.66)	2.75 (0.81)	3.67 (0.18)	3.58 (0.17)
FIRST DAY PLACEBO	2.50 (1.16)	4.83 (1.88)	3.50 (0.24)	3.00 (0.40)
SIX DAYS PLACEBO	3.11 (0.65)	5.20 (1.03)	3.42 (0.23)	3.56 (0.11)
0.16 mg/kg DIAZEPAM	1.67 (0.83)	2.17 (0.72)	3.67 (0.23)	3.50 (0.24)
0.40 mg/kg DIAZEPAM	2.50 (0.55)	3.67 (1.15)	2.83 (0.82)	2.67 (0.73)
0.80 mg/kg DIAZEPAM	3.50 (0.68)	7.00 (1.02)	2.39 (0.32)	2.17 (0.56)
1.60 mg/kg DIAZEPAM	5.00 (0.89)	9.83 (1.07)	3.50 (0.24)	1.83 (0.66)

There was a small, but persistent deterioration of performance on placebo days in relation to the no drug control days and diazepam doses were analyzed against the means of the last six placebo days in the one way ANOVAs of the four dependent measures. The analysis of habit errors produced a significant drug effect ($F_{4,20} = 4.100$, $p<.02$, but the only significant difference between means was between the 0.16 and 1.60 mg/kg doses, i.e., none of the drugs doses was significantly different from the placebo scores. (Had the no drug control days been used for the comparisons, the difference between the 1.60 mg/kg dose and the control days would also have reached significance. When total errors were examined, the drug effect was again significant ($F_{4,20} = 11.79$, $p< .01$). The Tukey tests indicated significant differences ($p< .01$) between placebo and the 1.60 mg/kg dose, between the 0.16mg/kg and both the 1.60 and 0.80 mg/kg doses, and between the .40 mg/kg and 1.60 mg/kg dose. There was no dose effect on Trial Two scores ($F_{2,40} = 1.95$, $p = .14$), and so object quality learning set performance was not affected in this study. As diazepam doses increased, there was a consistent decrease in group means for the Critical Trial Correct scores, but the significance was marginal ($F_{4,20} = 2.70$, $p = .06$).

F. Operant Performance:

Several operant schedules were evaluated for possible inclusion in the test battery. The sections which follow describe the schedules, give the relationships, if any, between performance on the schedules and social variables, and summarize the effects of drugs on performance on each. This part of the report concludes with summary of the advantages and disadvantages of each reinforcement schedule for the test battery.

DRL Schedules:

DRL procedure. Successful performance on differential reinforcement of low rate of response (DRL) schedules requires the animal to inhibit responding for a prescribed period of time in order to receive a reinforcer. The addition of a limited hold (LH) requirement, which provides an interval within which the animal must respond once it has waited through the initial delay period, increases the difficulty of the task. It was expected that the schedule would be sensitive to drugs which reduce response inhibition as well as those which have sedative effects. Performance also would be affected by drugs which interfere with whatever mechanisms the monkey might use to accurately meter the passage of time. The seven oldest males from NT-Troop were trained on a DRL-18 sec, LH-10 sec schedule. This required the monkey to delay 18 seconds between responses in order to receive a reinforcer; responding sooner than 18 seconds reset the timers and instituted another 18 sec delay. The limited hold required the animal make a response within 10 seconds once the 18 sec delay requirement had been met, otherwise no reinforcer was given.

Monkeys were allowed to earn 40 reinforcers (banana pellets) during each session; sessions were terminated after 60 min if the animals had not finished. Three measures of performance were obtained: Efficiency Index (EI) - the reciprocal of total responses made divided by the number of reinforcers obtained. An EI of .50 or larger indicates that the monkey is averaging two or less responses per reinforcer. (This is generally indicative of highly efficient performance on the schedule. However, when responding drops to a very low rate, such that the LH requirement is exceeded repeatedly, EI's may remain relatively high although it takes the monkey considerably longer to obtain 40 reinforcers.) Response Bursting - the 18 second interval was divided into six 3 second response bins and bursting was defined as the number of responses in the first bin (interresponse time <IRT> distributions were also obtained, these allow a study of response patterning across bins), and Limited Holds - the number of times the animal exceeded the limited hold

requirement during a session.

DRL performance and social behavior. In our previous work (Bunnell, 1982) we had found two relationships between DRL performance and social variables. During initial training on the schedule, the achievement of efficient performance on the schedule was positively correlated with high social rank. Then, once performance stabilized, response bursting was positively correlated with the frequency of aggressive responses exhibited by each monkey. This was demonstrated experimentally by removing and replacing animals of varying social rank in the groups and relating the changes in aggressive response frequencies produced by these manipulations to changes in DRL performance.

Five of the monkeys in the present experiments had participated in the earlier study and one of these, Weed, began retraining late because of illness. By June, 1984 the other four animals were performing well on the DRL-18 sec schedule while Weed and the two inexperienced animals were still on a less stringent DRL-9 sec schedule. Because of differences in stage of training, the efficiency ratios of all seven animals could not be compared directly. However, within each subset of animals, the highest ranking animals had the best efficiency indexes (EIs) within their groups:

DRL-18 sec			DRL-9 sec		
Animal	Rank	EI	Animal	Rank	EI
Barker	1	.53	Weed	3	.50
Eju	2	.43	Allen	5	.44
Tag	4	.13	Kukla	6.5	.35
Hobbit	6.5	.49			

After all seven animals were on a DRL-18 sec schedule, the rank order correlation between ER and social rank was +.65 at the time the first atropine sulphate study described below was run. This was despite the fact that three of the animals had not reached maximum efficiency at this time and that the EI data were taken from scores on placebo days when there may have been some carryover of drug effects. The correlation between rank and efficiency ratios on the 12 placebo days during the last atropine experiment rose to +.73 ($p < .05$). There had been a reorganization rank structure between the first and second atropine experiments.

Although Tag had the highest frequency of aggressive responses and showed the most response bursting during the placebo days of the first atropine experiment, the overall correlation between frequency of aggressive behavior and bursting was low. An experimental manipulation of the social

situation was conducted at the beginning of the second year of the project. Barker, the alpha male, was removed and this produced an increase in fighting among the remaining animals as well as a shift in the rank of some of the animals (details were reported earlier in the section on social behavior.) At this time, there was a marginally significant correlation of +.71 ($p = .05$) between frequency of aggressive responses and response bursting

As noted earlier, we have seen these same two correlations between social behavior and performance in the past and it is interesting that they keep appearing despite changes in rank, aggression, and performance. High ranking animals are generally the most efficient performers on this schedule and aggressive monkeys tend to show more response bursting than nonaggressive monkeys, whatever their rank.

Effects of caffeine on DRL performance. In the first experiment with caffeine, doses of 12, 4, or 0.8 mg/kg caffeine sodium benzoate were administered im 5 min before the beginning of testing. Placebo (physiological saline) days alternated with caffeine days and Mondays were warmup days during which saline was also given. The results are given in Table 33. (Saline scores are means across four days.) In order to examine the general effects of caffeine, the data from the monkeys on both the DRL-18 and DRL-9 sec schedules have been combined in the table. One-way within subjects ANOVAs of the combined data showed signficant effects for Efficiency Index ($F_{3,18} = 3.57$, $p < .05$) and Response Bursting ($F_{3,18} = 3.64$, $p < .05$) but not for the Limited Holds measure. With regard to the two different schedule requirements, we found that in three of the four monkeys on the DRL-18 sec schedule, the 12 mg/kg dose produced a dramatic increase in total responses that was characterized by bursting during the early part of the delay interval. This resulted in low Efficiency Indexes although all three animals obtained all 40 reinforcers during the 60 min allowed for the test. The drug produced no consistent changes in the frequency with which the animals exceeded the 10 sec limited hold requirement. The fourth monkey on the DRL-18 sec schedule had very high baseline reponse rates and showed only a slight increase in responding with this dose of the drug. This is consistent with the literature which suggests that there is an interaction between the effects of caffeine and baseline operant response rates such that response increases are best observed against a background of low basal rates. Similar increases in responding were seen in two of the three monkeys working on the DRL-9 sec schedule; the third animal was not affected by this or either of the lower doses. There was a reduction in the frequency with which two of these monkeys exceeded the 30 sec limited hold requirement, but the effect was small. At the lower doses in the rest of the monkeys, the effects decreased, although four of the animals were still

above baseline, even at the 0.8 mg/kg dose.

Table 33

Effects of Caffeine Sodium Benzoate on DRL Performance: Experiment One (Means +/- SEM)

	DOSE (mg/kg)			
	Saline	0.8	4	12
Efficiency Index	.41 (.05)	.37 (.09)	.37 (.08)	.22 (.06)
Response Bursting	6.39 (1.55)	6.74 (2.16)	7.99 (2.02)	11.79 (2.40)
Limited Hold Exceeded	39.50 (5.53)	36.29 (5.95)	36.86 (3.78)	32.00 (6.52)

From the literature, and from our own pilot observations of animals in the activity cage, we had expected to see a depression of responding at the 12 mg/kg dose, but found an increase instead. We therefore repeated the experiment, using doses of 36, 24, 12, 4, and 0.8 mg/kg caffeine sodium benzoate. For this experiment, all animals were on the DRL-18 sec 10-sec LH schedule. The results are given in Table 34; saline scores are means for five days.

Table 34

Effects of Caffeine Sodium Benzoate on DRL Performance Experiment Two (Means +/- SEM)

	DOSE (mg/kg)					
	Saline	0.8	4	12	24	36
Efficiency Index	.55 (.08)	.54 (.10)	.43 (.08)	.37 (.09)	.42 (.11)	.40 (.09)
Response Bursting	5.57 (1.98)	5.96 (2.60)	6.60 (2.15)	8.69 (2.53)	8.21 (2.99)	7.60 (2.41)
Limited Hold Exceeded	24.43 (5.50)	43.86 (11.36)	27.43 (10.63)	21.71 (4.81)	21.86 (4.77)	35.29 (11.18)

Baseline performance in the group improved in the second experiment, i.e., EIs increased, response bursting decreased, and the number of limited holds exceeeded was reduced. Five of the seven monkeys accounted for these changes. Of the other two, one (Hobbit) maintained a baseline EI of approximately .50 in both studies while the other (Tag) continued to respond at a high rate, exhibited a lot of bursting, and did not improve his

low EI. As was the case in the first experiment, the animals made more responses and were generally less efficient when given caffeine ($F_{3,30} = 2.78$, $p < .05$). Response bursting increased as well ($F_{3,30} = 2.48$, $p < .05$). EIs were lowest and bursting highest at the 12 mg/kg dose, suggesting the presence of the expected U-shaped relationship between dose and performance. The limited hold data did not reach significance; interpretaion of these scores was complicated by the unexpected increase at the 0.8 mg/kg dose. The high scores at the 36 mg/kg dose were consistent with the reduced responding reflected in the other two measures, however.

Individual differences in dose response curves were apparent in both experiments, although shifting the curves left or right revealed that the shape of the functions tended to be similar across subjects. Overall, the results of the second experiment confirmed those of the first experiment in all major respects and demonstrated the expected drop in responding in several animals at the higher doses. Some monkeys showed better performance at the lower doses, all (even Weed at the 36 mg/kg dose) exhibited lower EIs and increased bursting at one or more doses, and several showed an increase in the frequency with which the limited hold requirement was exceeded at the higher doses, indicating that responding was depressed below control levels.

Effects of atropine sulphate on DRL performance. The effects of atropine sulphate on DRL performance were investigated in the NT-Troop males. In the first experiment, doses of .40 mg/kg (given twice to each monkey), .20 mg/kg (also given twice), and .08 mg/kg (given once) were compared to performance scores averaged across six days on which physiological saline was administered. The order of atropine doses was: .40, .08, .20, .20, and .40 mg/kg. All monkeys were on the DRL-18 sec, LH-10 sec schedule. The results are presented in Table 35. Atropine sulphate produced a substantial disruption of performance at the two higher doses and a somewhat more variable decrement at .08 mg/kg. The animals began testing 15 min after being given the drug; had we waited longer before beginning the test sessions, performance probably would have been even worse, since there was evidencs of a progressive failure to respond later in the sessions. The total number of reinforcements received during the sessions is given at the bottom of the table. Generally, animals that began responding quickly and efficiently under placebo conditions earned more reinforcers under the drug than those that were more dilatory baseline responders. This appeared to be due to the gradual onset of drug effects as the tests probably began before the maximum effects had been reached. There also was some suggestion that tolerance was developing as the experiment progressed, but the confounding of dose and order of administration of the different doses in conjunction with

individual differences in response to the drug makes this difficult to assess. However we did compare the Efficiency Indexes for the two administrations of the .20 and .40 mg/kg doses and found that the repetitions effect was not significant ($F_{1,6} = 3.61$, $p > .10$); (also, there was no dose effect and no dose by repetition interaction). The data in Table 35 use the means of the two administrations of the two highest doses.

Table 35

Effects of Atropine Sulphate on DRL Performance (DRL-18 sec; LH-10 sec); Mean (+/- SEM)

	Dose (mg/kg)			
	Saline	.08	.20	.40
Efficiency Index	.40 (.06)	.29 (.09)	.26 (.05)	.21 (.03)
Response Bursting	6.10 (1.54)	6.29 (1.45)	5.76 (1.37)	5.91 (1.54)
Limited Hold Exceeded	61.71 (10.37)	126.29 (14.37)	108.71 (8.23)	115.86 (14.48)
Reinforcers Received	34.04 (2.07)	21.29 (5.92)	17.93 (5.16)	14.57 (4.55)

One-way ANOVAs of the data revealed significant effects of atropine sulphate on the efficiency index ($F_{3,18} = 3.69$, $p < .05$), number of times the limited hold was exceeded ($F_{3,18} = 13.15$, $p < .001$) and total number of reinforcers received ($F_{3,18} = 7.69$, $p < .01$). The monkeys were impaired on the three measures of performance at all three doses. There was no effect on response bursting at any dose, however.

Although all of the monkeys except Tag routinely earned 40 reinforcements per daily session before the experiment began (Tag averaged about 35), only Hobbit and Weed obtained 40 reinforcements on all six saline days during the experiment, indicating a carryover of drug effects on some placebo days. There were considerable individual differences in the patterning of these carryover effects. One animal, Kukla, consistently failed to earn 40 pellets on placebo days after atropine days; this effect was unrelated to dose. Eju missed between 1-9 pellets on saline days following the first four drug days, but received 40 after the second administration of .40 mg/kg. Two animals were affected early in the experiment: Tag received only three pellets on the day after the first .40 mg/kg dose, but earned 40 on all other saline days while Barker was affected by the first doses of .40 and .20 mg/kg but not by

.08 or the second administrations of the two higher doses. Allen failed to complete the session on the days following the .08 and the second .40 mg/kg doses, but earned 40 pellets on the other saline days.

Because of our concern that the 15 min delay between drug administration and the start of testing may have been too short, the next experiment repeated the .20 and .08 mg/kg doses, but utilized a 30 min delay. This study, conducted 5 weeks after the initial experiment, utilized one presentation of each of the two doses of atropine sulphate and three placebo days. Table 36 compares the Efficiency Index, Limited Hold, and Reinforcers Received data from this study with the study that used the 15 min delay (Table 35) for these two doses.

Table 36

Comparison of Atropine Sulphate Effects on DRL Performance for Delays of 15 and 30 min between Injection and Testing (Means +/-SEM)

	Dose (mg/kg)					
	Saline		.08		.20	
Delay (min.)	15	30	15	30	15	30
Efficiency Index	.40 (.06)	.42 (.06)	.29 (.09)	.34 (.04)	.26 (.05)	.18 (.04)
Limited Hold Exceeded	61.71 (10.37)	26.81 (5.25)	126.29 (14.37)	46.83 (12.58)	108.71 (8.23)	118.71 (13.50)
Reinforcers Received	34.04 (2.07)	39.50 (0.42)	21.29 (5.92)	32.57 (5.69)	17.93 (5.16)	20.14 (5.33)

The monkeys improved their scores on both limited holds and reinforcers received in the five weeks between the two studies. (The data for no drug days obtained at this time show that the differences in saline scores were not caused by the saline injections.) Since delay intervals and repetitions were confounded, the delay data must be examined in terms of proportional changes in performance relative to the saline data for each experiment. When this is done, there is little or no difference in drug effects for the two delay intervals at the .08 mg/kg dose. However, performance after atropine (both doses combined) was relatively much worse on the efficiency index and limited hold scores (but not reinforcers received) at the 30 min delay. It was decided to employ the 30 min delay between injection and testing in the last experiment in this series.

The third experiment, begun 12 weeks after the second study, examined the effects of atropine sulphate and atropine methyl nitrate on DRL performance. Atropine methyl nitrate, the

quarternary nitrogen derivative of atropine, is presumed to have largely peripheral effects as a muscarinic receptor blocker since it does not pass the blood brain barrier readily (Weiner, 1980). It has been reported that it does not have much effect on most behavioral and EEG measures (Russell, 1982) when compared with atropine sulphate. The seven NT-Troop males were used again with doses of .20, .08, and .032 mg/kg of both drugs. (The lower dose was added to examine the lower end of the dose response curve.) Placebo (physiological saline) days alternated with drug days with Mondays always being placebo days. The order of administration was .08, .20, and .032 mg/kg of one compound followed by the same order for the other compound. Four monkeys received the atropine sulphate (AS) series first followed by the atropine methyl nitrate (AMN) series. The other three monkeys received AMN first. There were a total of 6 drug days and 12 placebo days in the experiment. The data are presented in Table 37.

TABLE 37

Effects of Atropine Sulphate (AS) and Atropine Methyl Nitrate (AMN) on DRL Performance (Means +/- SEM)

		DOSE mg/kg					
	Saline*	.032		.08		.20	
		AS	AMN	AS	AMN	AS	AMN
Efficiency Index	.46 (.06)	.46 (.06)	.36 (.05))	.30 (.05)	.22 (.05)	.19 (.05)	.22 (.04)
Response Bursting	5.46 (1.26)	5.57 (1.65)	5.44 (1.38)	6.01 (1.54)	5.50 (1.96)	4.64 (1.91)	4.90 (1.54)
Limited Hold Exceeded	31.14 (8.30)	38.43 (14.73)	74.00 (13.46)	80.57 (14.84)	109.14 (16.32)	106.86 (20.90)	97.43 (17.44)
Reinforcers Received	39.11 (0.73)	38.14 (1.86)	33.43 (2.53)	33.71 (2.66)	22.57 (6.08)	14.71 (6.73)	20.57 (7.03)

*Scores for Saline days are the means of 12 sessions.

As was the case in the first two experiments, the two-factor (drug and dose) within subjects ANOVAs revealed significant effects of the drugs on the efficiency index, the number of limited holds exceeded, and the total number of reinforcers received (out of a possible 40). Of greatest interest were the significant drug by dose interactions which appeared in all three analyses. For EI, the interaction had an $F_{3,18} = 3.40$, $p < .05$; for limited holds it was $F_{3,18} = 3.96$, $p < .05$, and for reinforcers it was $F_{3,18} = 6.27$, $p < .01$. Once again, there was no effect on response bursting. Somewhat surprisingly, post hoc Tukey tests of the cell means

indicated that performance decrements appeared first at lower doses of AMN than for AS. At the .20 mg/kg dose, the slightly poorer performance under atropine sulphate was not significantly different from the atropine methyl nitrate scores on any of the three measures. The saline day scores for the second and third experiments were quite similar and there were no differences in the atropine sulphate scores at either dose, except for the limited holds scores at the .08 mg/kg dose. Limited holds were exceeded much more frequently in the third experiment. It is possible that some drug tolerance was present in the second experiment and that it had disappeared 12 weeks later.

There was a drop in the efficiency scores which was not accompanied by an increase in bursting. This, together with the increase in limited holds exceeded and a reduction in the number or reinforcers received, indicate that the atropines produced both a general slowing of responding and a loss of temporal patterning of responses. Based on our initial study of atropine effects on activity and ingestive behavior with the C-Troop males, we do not think it likely that the primary effects of atropine on DRL performance were the result of drug induced thirst causing the monkeys to reduce their responding for the dry banana pellets. The effects of even the lowest dose of atropine methyl nitrate were muuch greater than we had been led to expect from the literature. The poorer performance under atropine sulphate as compared to atropine methyl nitrate at the .20 mg/kg doses suggests the operation of a central effect of atropine at this relatively large dose. At the .08 and .032 mg/kg doses, peripheral effects might have produced the deterioraton in performance. It is possible that a selective central effect also may have been present, since deficits tended to be smaller with low doses of atropine sulphate and there were some instances where performance was actually better than mean performance on placebo days. However, we do not know whether the time between the administration of the two drugs and their production of peripheral effects could be sufficiently different to account for the data.

There was no discernible interaction between performance with any dose of either atropine and social variables in these experiments. In other words, no score on any social variable, including rank, was related to the nature or magnitude of performance changes induced by the drugs.

Effects of diazepam on DRL performance. The effects of diazepam on DRL performance were examined in six NT-Troop males. One animal had died between the end of the last atropine study and the beginning of the diazepam experiment. Training of a replacement had begun, but the new monkey's baseline performance was not comparable to that of the other six. Scores with i.m. doses of 0.16, 0.40, 0.80, and 1.60 mg/kg are

compared with performance on both vehicle days (means of five days) and no drug days (means of five days) in Table 38. The diazepam dose order was 0.80, 0.16, 1.60, and 0.40. There was a two day separation between the administration of the 0.16 and 1.60 mg/kg doses to minimize the possible behavioral effects of the metabolites of diazepam. All other doses of diazepam were given at least six days apart.

TABLE 38

Effects of Diazepam on DRL Performance. Means (+/- SEM)

			DOSE mg/kg			
	No Drug	Vehicle	.16	.40	.80	1.60
Efficiency Index	.55 (.05)	.45 (.03)	.54 (.06)	.58 (.05)	.42 (.11)	.48 (.05)
Response Bursting	16.70 (4.07)	28.87 (5.26)	20.83 (4.81)	12.83 (3.48)	18.50 (6.57)	21.17 (4.32
Limited Hold Exceeded	3.88 (0.72)	27.87 (5.96)	18.67 (7.99)	17.83 (8.52)	83.17 (16.02)	21.17 (7.06)

The vehicle used as the placebo for the diazepam injections interfered with performance on the DRL schedule. (The vehicle contained 40% propylene glycol, 10% ethyl alcohol, 1.5% benzyl alcohol and 5% sodium benzoate and benzoic acid in Water for Injection.) The within-subjects ANOVAs which compared the mean performance on the 5 no drug days with those of the 5 vehicle days were significant on all three measures: $F_{1,8} = 27.00$, $p < .01$ for EI; $F_{1,8} = 7.69$, $p < .05$ for response bursting, and $F_{1,8} = 17.09$, $p < .01$ for limited holds exceeded. An examination of performance across vehicle days indicated that scores did not change very much, indicating that neither tolerance nor sensitization developed in response to repeated administrations of vehicle alone. As can be seen from Table 38, performance under diazepam is generally intermediate between no drug day scores and vehicle days. On the EI, performance was similar to that on no drug days for the two lowest doses of diazepam and similar to the vehicle days for two higest doses. All monkeys had increases in their limited hold scores at the .80 mg/kg dose. Failures to respond within the 10 sec limited hold interval increased by from 2.5 to 22 times the usual scores and three animals failed to obtain all 40 reinforcers within the alloted 60 min. With the exception of one monkey on one vehicle day, this was the only time during the entire experiment that all animals did not finish the test. While limited hold scores were elevated at all doses of diazepam, the increases seen with the other doses has to be attributed to vehicle effects in the absence of additional information. The .80 mg/kg dose was the first experience these

monkeys had with diazepam; we would have expected that sedative effects of the 1.60 mg/kg dose would have produced even more striking increases in Limited Hold scores; however, this was not the case and the monkeys performed relatively efficiently at this dose. (The 1.60 mg/kg dose was the monkeys' third experience with diazepam and there were three vehicle days plus the .016 mg/kg day intervening between the .80 and the 1.60 mg/kg doses.)

Because of the effects of vehicle on performance, diazepam effects on this task are difficult to interpret. However, with the exception of the first administration of a moderately large dose (0.80 mg/kg) there is no suggestion that diazepam itself seriously interfered with performance on this relatively difficult task. (Vehicle effects are something else, of course.) If anything, the finding that diazepam scores were often intermediate between scores on no drug days and vehicle days suggests that the drug may partiallly ameliorate the effects of the vehicle injections on performance, perhaps because of its anxiolytic properties. It should be noted that the volume of vehicle was never more than .16 ml/kg, therefore the effects are probably due to an interaction between propylene glycol and alcohol.

Fixed Interval Schedules:

Fixed Interval procedure. On a fixed interval (FI) schedule of reinforcement, the animal receives a reinforcer for a response made after a preestablished time interval has passed. In well trained animals, the cumulative response curves on this schedule are scalloped, that is, there is a pause after a rewarded response followed by a gradual increase in responding as the end of the next interval approaches. Scalloping may be identified by comparing response frequencies early in the interreinforcement interval with those late in the interval and it can be quantified by calculating an Index of Curvature (IC) (Fry, Kelleher and Cook, 1960). Performance on FI schedules has been shown to be sensitive to caffeine (e.g. Stinnette and Isaac, 1975; Meliska and Brown, 1982). For this reason, and because we had seen a relationship between performance on this schedule and social variables in our earlier work with rhesus monkeys (Bunnell, et al, 1979a,b) the I-Troop males were placed on a FI schedule and the effects of cafeine sodium benzoate and atropine sulphate were examined.

A FI-30 sec schedule was used in which the monkey received a reinforcer (banana pellet) for a response after 30 seconds had passed since its last reward. The 30 sec interval was divided into six 5 sec bins and response frequency in the first bin following reinforcement was used as a measure of response bursting. Other performance measures obtained were the ratio of responses to reinforcers received and the total number of

reinforcers received. (The maximum number was 40; if the monkey had not earned 40 pellets at the end of 60 min the session was terminated.)

The eight I-Troop males began training at the end of March, 1984. Training was very slow and, by mid-June, the majority of the animals had progressed only to a FI-20 sec schedule; although they were earning all 40 pellets during a session, they showed little evidence of the response curve scalloping which is indicative of temporal discrimination and efficient performance on the task. By mid-August, 6 of the animals were shifted to a FI-30 sec schedule and a 7th was put on this schedule two weeks later. The 8th monkey, Alabama, who ranked second in the social hierarchy, did not perform consistently and is excluded from the data presented below. By mid-September, the seven animals were showing good scallops in their response curves, i.e., the monkeys had positive indexes of curvature indicating that the majority of responses were occurring in the last 15 sec of the 30 sec interval.

Effects of caffeine on FI performance. A pilot experiment, conducted in June, 1984 during training and before performance had stabilized, utilized doses of 0.8, 4, and 12 mg/kg caffeine sodium benzoate alternated with placebo (physiological saline) days. The daily sessions were started 5 min after im injections of the drug. At the 12 mg/kg dose, response rates increased in 3 monkeys, decreased in 3, and were unchanged in the 7th animal. There were no obvious effects at the two lower doses. In the main experiment, conducted in conjunction with the experiment on atropine described in the next section, doses of 4, 12, and 36 mg/kg of caffeine were administered 5 min before testing on a FI 30-sec schedule. The results are presented in Table 39. Saline scores are the means +/- SEM for the 12 placebo days of the experiment. The dependent measures were the presence (positive IC) or absence of scalloping, number of first bin responses (a measure of response bursting), mean responses/reinforcer received, and total number of reinforcers received.

There was a dose related loss of response scalloping such that none of the 7 monkeys had positive ICs at the 36 mg/kg dose; scalloping was present in 3 of the animals at the 12 mg/kg dose and in 6 at 4 mg/kg. This was accompanied by increased first bin responding at 36 and 12 mg/kg ($F_{3,12}$ = 5.04, $p < .02$). (In 3 animals increases in responding were greater at 12 mg/kg than at 36mg/kg, suggesting the presence of the inverted U-shaped dose response curve relationship described earlier. However, since the larger dose was given last, these changes could be tolerance effects.) Most animals showed an increase in their response/reinforcement ratios at one or more dose levels ($F_{3,12}$ = 3.81, $p < .05$). The results are consistent with those from other laboratories in

that increases in response rate occur on this schedule at some doses of caffeine. However, the decreased temporal discrimination evidenced by the loss of scalloping has not been reported in the other studies (e.g. Mechner and Latranyi, 1963; Davis, Keensler and Dews, 1973).

Table 39
Effects of Caffeine Sodium Benzoate on FI Responding (FI-30 sec) Means (+/- SEM). (Placebo Data are Means for 12 days).

	Dose(mg/kg)			
	Saline	4	12	36
Scalloping (# monkeys with positive ICs)	7/7	6/7	3/6*	0/7
First Bin Responses	5.2 (1.0)	5.2 (1.2)	6.9 (1.7)	7.2 (1.0)
Responses/ Reinforcement	2.5 (0.4)	2.5 (0.4)	3.9 (1.1)	3.7 (0.6)
Reinforcers Received	40.0 (0.0)	39.7 (0.3)	38.4 (1.6)	39.2 (0.8)

*One animal was ill on the day the 12 mg/kg dose was given and was not tested at this dose.

Effects of atropine on FI responding. The first part of this experiment was done in association with the study of caffeine effects described in the preceding section. There were two replications of .20 mg/kg atropine sulphate, one with 15 min and one with 60 min between drug administration and the beginning of testing, and three of .08 mg/kg, one with a 15 min and two with a 60 min delay. The order of administration was: .20 - 15 min delay; .08 - 15 min delay; .20 - 60 min delay; .08 - 60 min delay; .08 - 60 min delay. The data are shown in Table 40. (Means of the two .08 mg/kg - 60 min delay days are used in the table.)

Table 40

Effects of Atropine Sulphate on Fixed Interval Responding
Means (+/- SEM)

	Dose (mg/kg)				
	Saline	.08		.20	
Delay (min):		15	60	15	60
First Bin Responses	5.37 (0.81)	5.84 (1.57)	6.97 (1.57)	3.47 (0.93)	2.91 (0.65)
Responses/ Reinforcer	2.71 (0.41)	2.53 (0.59)	4.26 (1.07)	2.64 (0.57)	3.04 (0.25)
Reinforcers Received	38.86 (0.81)	40.00 (0.00)	31.79 (3.07)	17.29 (4.19)	11.86 (3.32)

There was a significant dose effect on first bin responses ($F_{2,12} = 6.62$, $p < .02$). Post hoc Tukey tests revealed that the difference was between the .08 and .20 mg/kg doses - neither atropine dose differed from the saline scores. This suggests the presence of an inverted U function, with the smaller dose perhaps producing a small increase in response bursting and the larger a substantial suppression in first bin responding. Neither the delay between injection and testing nor the interaction between delay and dose were significant. Neither delay interval nor dose had an effect on the number of responses made for each reinforcer obtained, but the .20 mg/kg dose caused the animals to respond slowly and they did not obtain all 40 reinforcers during the 60 min test sessions ($F_{2,12} = 63.82$, $p < .001$). The delay between injection and testing was also significant on this measure, with the monkeys doing worse with the 60 min delay ($F_{1,6} = 18.55$, $p < .01$); there was no interaction. At the dose of .20 mg/kg, response scalloping reappeared in three animals with the 60 min delay; this was probably the result of a drop in overall responding rather than a reestablishment of temporal discrimination. With the .20 mg/kg dose, despite the overall decline in responding, responses per reinforcement increased in six of the monkeys at at least one delay interval. The animals appeared to have lost the ability to make a single, discrete response when they did respond. With the first 60 min delay at the .08 mg/kg dose, scalloping was present in 6 of 7 monkeys, but was lost in five of these animals with the replication. Increased response bursting, accompanied by a trend toward increased responses/reinforcement, was seen in five monkeys on either the first or second administration of this dose with the 60 min delay. At this dose and delay interval, two animals failed to complete the session on the first administration and five failed on the second administration. This, and the changing patterns of first bin responses suggest the possibility of

behavioral sensitization to the drug.

In a brief followup study, we used doses of .032 and .20 mg/kg atropine sulphate in order to look at the lower end of the dose response curve and to see if we could substitute a 30 min delay between injection and the start of testing for the 60 min delay used in the first experiment. Performance under the .20 mg/kg dose was severely disrupted with the 30 min delay. The findings were very similar to those seen at the 60 min delay interval in that the monkeys performed slowly and did not finish the sessions, temporal discrimination was lost, and first bin responding was decreased. Individual differences in the animal's responses at the 30 min delay were apparent in that some monkeys' performance was similar to their behavior following the 15 min delay while the resoonse patterns of others were more similar to those they exhibited with the 60 min delay. Thus, it appears that there are individual differences in the rapidity with which atropine affects performance. The .032 mg/kg dose of atropine sulphate had little or no effect on performance. This completed testing on the FI schedule - the schedule was used primarily to validate the caffeine effects on FI performance that have been reported by other investigators - and the I-Troop monkeys were then trained and tested on a random interval schedule as described in the next section.

Random Interval Schedules:

Random interval procedure. Upon completion of testing of the effects of atropine sulphate on FI performance, the I-Troop males were retrained on a random interval (RI) schedule. On this schedule, a random interval-1 min schedule (RI 1-min), responses are reinforced on the average of once per minute, but the actual intervals between the availability of reinforcers are produced by a random interval generator. Such a schedule tends to produce a moderately high and constant rate of responding during the intervals and this provides a good background against which to assess increases and decreases in response rates and frequencies as a function of drug administration. The monkeys were allowed to earn 40 banana pellet during daily sessions lasting 60 min. In recording the animals' responses, the random intervals were divided in 12 sec bins. Performance measures included Total Responses, Responses per Reinforcement, proportion of total responses in the first bin (a measure of Response Bursting), and the number of Reinforcements Received out of a possible total of 40 in the test session. As a variant of this schedule, reinforcement was randomly omitted 10 percent of the times the monkey completed the schedule requirements - this was done by dropping the banana pellet through the bottom of the food hopper before the monkey could reach it. The omission of reinforcement procedure was designed to frustrate the animal and produce response

bursting in the first bin of the post omission interval.

RI performance and social behavior. No significant relationships were found between any of the performance measures on the RI schedule and social variables. Based on our earlier work (Bunnell, 1982 and Bunnell, et al, 1979 a,b), we had expected to find a correlation between response bursting and social rank in the group. Although there was some response bursting in response to the omission of reinforcement, the magnitude of the effect was small (see below). One high ranking animal (Alabama) failed to reach stable performance on the schedule.

Effects of atropine on RI performance. Seven of the 8 males in I-Troop had reached stable performance on the RI-1 min schedule by June, 1985 (the eighth performed poorly and was not included in these experiments) and testing of the effects of atropine sulphate and and atropine methyl nitrate was begun. Doses were .032, .08, and .20 mg/kg and the interval between drug administration and the start of testing was 30 min. The first part of the experiment was run with the animals on 100% reinforcement; the second was done with random omission of reinforcement on 10% of the completed intervals. Sessions were terminated after an animal earned 40 banana pellets or at the end of 60 minutes under both reinforcement conditions. Drug days were alternated with placebo (physiological saline) days with some monkeys receiving AS and the rest AMN on a given drug day. The order in which the doses were administered was .08, .20, and .032 mg/kg; this order was then repeated so that all animals received all doses of both drugs under the 100% reinforcement condition. During testing with omission of reinforcement, the order of the doses was the same, but the monkeys received a given dose of both drugs before being tested on the next dose; once again, drug days were alternated with placebo days. One monkey became ill during testing with omission of reinforcement and he was removed from the experiment while undergoing treatment. Means for the 100% reinforcement condition are shown in Table 41a; Table 41b gives the same information for the 90% reinforcement condition.

The 90% reinforcement condition did not produce much response bursting. Although all six monkeys made more first bin responses under the 90% reinforcement condition than they did at 100% reinforcement on placebo days ($F_{1,5} = 11.01$, $P < .05$), the proportion of first bin responses to total responses was not significantly increased.

In the 100% reinforcement condition, there were dose dependent decrements in performance under both drugs. Total responses ($F_{3,15} = 5.44$, $p < .01$), responses/reinforcer ($F_{3,15} = 4.07$, $p < .05$) and reinforcers received ($F_{3,15} = 9.28$, $p < .01$) decreased markedly with the .20

mg/kg doses of both drugs. There was no effect on response bursting. Under the 90% reinforcement condition, much the same trends are present, but, due to the increased responding on the .08 mg/kg days of both drugs, only the reinforcers received measure was statistically significant ($F_{3,15} = 8.16$, $p < .01$).

Table 41
Effects of Atropine Sulphate (AS) and Atropine Methyl Nitrate (AMN) on RI 1-min Performance: a. 100% Reinforcement; b. 90% Reinforcement. Data are Means (+/-)SEM.

		Dose(mg/kg)					
		.032		.08		.20	
Drug:	Saline	AS	AMN	AS	AMN	AS	AMN
a. 100%:							
Total Responses	720.0 (259.6)	663.1 (265.4)	667.1 (257.1)	551.0 (274.0)	331.3 (154.0)	249.1 (124.9)	275.4 (198.5)
Responses/ Reinforcer	18.5 (6.5)	16.6 (6.6)	17.2 (6.4)	14.4 (6.7)	9.6 (3.6)	7.5 (2.9)	9.0 (4.7)
Response Bursting	.26 (.06)	.28 (.06)	.30 (.06)	.31 (.06)	.29 (.05)	.39 (.06)	.25 (.06)
Reinforcers Received	39.9 (.09)	40.0 (0.0)	40.0 (0.0)	33.0 (4.6)	27.7 (4.8)	20.9 (6.2)	22.3 (6.1)
b. 90%:							
Total Responses	654.0 (235.7)	492.8 (216.4)	483.3 (206.1)	700.8 (290.8)	619.2 (355.9)	160.0 (75.3)	354.7 (189.2)
Responses/ Reinforcer	13.4 (4.4)	17.6 (10.6)	10.8 (4.6)	22.6 (10.5)	21.6 (8.3)	13.5 (6.5)	17.0 (8.7)
Response Bursting	.29 (.08)	.37 (.08)	.35 (.08)	.21 (.05)	.28 (.06)	.24 (.06)	.24 (.03)
Reinforcers Received	39.9 (0.1)	40.0 (0.0)	40.0 (0.0)	36.5 (3.5)	28.2 (5.9)	21.5 (8.1)	22.2 (6.2)

Diazepam Effects on RI Performance. The effects of doses of 0.16, .80 and 1.60 mg/kg diazepam were studied using six of the I-Troop males. A seventh male was also tested but, because he did not finish the tests on several no drug and placebo days, his data have been excluded from the analyses. (We also had intended to use a dose of 0.40 mg/kg in this

experiment, but following the shutdown of the laboratory during the Christmas break, we had to change the testing schedule due to a major upheaval in the social structure of the group which resulted in severe disruptions of performance. However, the 0.40 mg/kg dose was used in the study of multiple RI schedules described in the next section of the report.) There were three days on which neither drug nor placebo were given, three placebo (diazepam vehicle) days, and the three drug days. The order of diazepam administration was 0.80, 0.16, and 1.60 mg/kg. There was a minimum of 48 hours between diazepam days. Reinforcement probability was 100% throughout the experiment. The results are presented in Table 42.

Table 42

Effects of Diazepam on RI-1-min Performance under 100% Reinforcement
Means (+/-)SEM.

	Dose(mg/kg)				
	No Drug	Vehicle	0.16	0.80	1.60
Total Responses	553.2 (184.7)	589.8 (228.7)	670.2 (238.8)	784.0 (168.7)	502.3 (136.9)
Responses/ Reinforcer	13.9 (4.6)	15.0 (5.6)	16.8 (6.0)	19.6 (4.2)	12.6 (3.4)
Response Bursting	.31 (.04)	.27 (.06)	.27 (.06)	.26 (.03)	.22 (.06)
Reinforcers Received	39.3 (.34)	38.2 (1.16)	39.8 (.17)	40.0 (.00)	39.7 (.33)

Most of the monkeys completed all of the tests under all conditions and earned 40 banana pellets within 60 min each day. Two animals failed to finish the task within 60 min on all three vehicle days, one of these also earned just 39 pellets on the day he received the 0.16 mg/kg, and a third monkey received 38 pellets under the 1.60 mg/kg dose. Two animals missed one or two pellets on one of the no drug days. Two animals had relatively high rates of responding on both no drug and placebo days (mean total responses ranged from 1105 to 1441) three had moderate rates (270 to 439) and one had low baseline rates (73 and 133). This produced the relatively large standard errors in the Total Response and Responses/Reinforcer measures in Table 42. Because of this, square root transformations ($x + 0.5^{1/2}$) of the Total Responses and Responses/Reinforcer data were used in the one way within subjects ANOVAs.

Diazepam produced a dose dependent increase in total responses at the two lower doses while responding at the 1.60

mg/kg dose was slightly depressed ($F_{4,20} = 3.69$, $p < .05$). The responses/reinforcer data reflect the same trend ($F_{4,20} = 3.56$, $p < .05$). Post hoc Tukey tests indicate that the significant differences were related to the differences in performance between the 0.80 and 1.60 mg/kg doses - it appears that the 1.60 dose has a sedative effect.

Punishment and RI schedule performance. One of the assigned tasks for the project was to examine the effects of electric footshock on performance with an eye toward incorporating a task involving shock (a physical stressor) into the test battery. Early in the project we ran a pilot study, using the C-Troop males to ascertain the appropriate shock intensities to be used on a free operant avoidance (Sidman avoidance) task scheduled to be conducted with the T-Troop males. We found that these animals quickly learned to make various behavioral adjustments which enabled them to minimize the effects of the shock. Subsequent attempts to get the animals to respond to avoid shock were not successful. It appeared that it would be necessary to place the animals in restraint chairs to insure that they would receive approximately the same amount of shock on each occasion and to get them to attend to the test situation. Keeping the animals chronically restrained was not practical both because we had access to only four chairs and needed to test at least six monkeys and because chairing the animals would disrupt the social testing.

As an alternative to the free operant avoidance task, we decided to look at response suppression to footshock. The I-Troop males that were trained on the RI 1-min schedule were used with the .90 probability of reinforcement schedule such that the animals received a footshock instead of a food pellet approximately 10% of the times the schedule requirement was met. Initially, the intensity of the brief (0.5"), constant current footshocks was set at 0.8 ma. Subsequently, as the animals became accustomed to the situation, the intensity was increased to 1.0 and 1.2 ma. Due to the operation of the random probability generator, monkeys received from 0 to 9 shocks during a session; the median shock frequency during a session was 4. A test session was terminated when a monkey had received 40 food pellets or after 60 min. Over a period of three weeks the monkeys were given shock on 5 of the 15 test days with the shock intensity set at 0.8 ma; this was followed by one week in which they received 2 shock sessions with the intensity at 1.0 ma. In all of these tests, the first day of each week was always a no-shock day and shock and no-shock days were alternated during the remainder of the week. By the fourth week, some of the animals were adapting to the shock, and no shock was given during the five sessions of the fifth week. In the sixth week, the animals received 0.8 ma shock on the third day and 1.2 ma on the fourth day. Although there was

considerable individual variation, all animals showed response suppression during the shock sessions, either in terms of a reduction in overall response frequency, a reduction in responding immediately following a shock, or both. In some cases, responding was attenuated to a point where an animal failed to receive all 40 reinforcers in the allotted 60 min. While it was possible to produce greater reductions in responding by increasing the duration and/or intensity of the shocks, we settled on the 1.2 ma intensity and the 0.5" duration for the drug study described in the next section.

Diazepam Effects on Punished RI Performance. The effects of doses of 0.16, .80 and 1.60 mg/kg diazepam on RI performance when the animals received footshock were studied using the seven I-Troop males. A shock intensity of 1.2 ma was used throughout the experiment which was conducted over a period of three weeks. Mondays were always control days on which the animals received neither shock nor drugs. Animals were shocked on the .90 probability schedule described earlier on three days a week. Each week, one of the shock days was a diazepam day, one was a vehicle day, and on the third shock day, they received no injection of any kind. The schedule was staggered so that different animals got drug or placebo on different days.

Five of the seven monkeys failed to complete their tests within 60 min on the shock days when neither drug nor placebo were given. A comparison of reinforcers received on shock (mean = 22.8) and no-shock control days (mean = 38.8) for all animals was significant ($F_{1,4} = 7.44$, $p < .05$). A comparison of the transformed (square root transformation) response frequency data (mean shock = 17.0; mean no-shock = 22.0) for these conditions was not significant, however $F_{1,4} = 2.93$, $p < .14$). The two monkeys that completed all the tests showed response suppression only on the first shock day. The diazepam data are presented in Table 43; the response frequency data are square root transformations.

The means for both measures showed a dose dependent reduction in response suppression to footshock. However, the within subjects ANOVAS produced a significant effect in the response frequency data ($F_{4,24} = 3.58$, $p < .02$) but not for reinforcers received ($F_{4,24} = 2.30$, $p < .10$). On this measure, the placebo scores were more like the low dose diazepam scores than the shock control (no injection) scores. The monkeys were inconsistent in their performance across drug and placebo doses and it appears that adaptation to shock and drug dose were probably confounded.

Table 43

Effects of Diazepam on Punished RI 1-Min Responding (Means +/- SEM)
(Response Frequency Data are Square Root Transformations)

		DOSE mg/kg			
	Control	Placebo	0.16	0.80	1.60
Response Frequencies	17.0 (5.7)	21.8 (5.6)	22.4 (6.1)	25.1 (6.2)	27.7 (5.3)
Reinforcers Received	22.8 (6.1)	29.0 (4.2)	30.6 (6.1)	30.3 (4.7)	39.4 (0.6)

The data were further analyzed by grouping the individual animal's scores on the diazepam days when they showed the smallest suppression to footshock and comparing the realigned response frequency data to the placebo and no-injection means. The realigned mean = 29.0 contained 4 scores from the 1.60 mg/kg, 2 from the 0.80 mg/kg and 1 from the 0.16 mg/kg doses. The ANOVA resulted in a $F_{2,12} = 11.40$, $p < .01$. The post hoc comparisons showed that the diazepam mean differed from the no-injection ($p < .01$) and placebo ($p < .05$) means, but that the no-injection and placebo means did not differ from each other.

MULT RI RI and RI EXT Schedules:

MULT RI RI and RI EXT procedure. During the last few months of the project, six I-Troop males were put on a multiple RI 1-min RI 1-min operant schedule in which a change in stimulus conditions was used to discriminate the two RI components. This was followed by a multiple RI 1-min extinction (EXT) schedule. The objective was to produce increased response rates in the first component of the schedule as a consequence of nonreinforcement during the second component. This effect, known as "positive behavioral contrast", might be a useful measure for studying drug effects on experimentally induced increases in response rates. Also, we were interested in the possible relationship between contrast effects and frequencies of aggressive behaviors.

The standard procedure for demonstrating behavioral contrast utilizes three stages; in the first, the animals receive the MULT RI RI schedule; this is followed by the MULT RI EXT condition which should produce positive contrast. Finally, the animals are returned to the MULT RI RI schedule with the expectation that response rates in the first component will return to baseline. The last stage is used to make certain that changes in the first component seen during the RI EXT stage are due to schedule interaction and not to the length of time the animals have been exposed to testing (Schwartz and Gamzu, 1977).

Six I-Troop males were retrained on the RI 1-min schedule in the fall of 1986 and then placed on the MULT RI 1-min RI 1-min schedule. The 54 min test sessions were divided into 9 6-min bins. First and second components alternated such that the first component occurred 5 times and the second 4 times in each session. This arrangement insured that the monkeys would always be reinforced during the last 6 min of the tests on extinction days. During the second component, a red cue light was illuminated next to the manipulanda. Because the number of reinforcers available in each bin of the RI schedules varied, The total pellets available to each animal als. varied. On average, each monkey earned about 45 reinforcers per session, although the range extended from a low of 32 to a high of 60.

Two weeks after responses had stabilized on the MULT RI RI schedule, the MULT RI EXT schedule was introduced for three consecutive days followed by two days of MULT RI RI and another MULT RI EXT day. Over the following three weeks, various combinations of alternations of RI RI and RI EXT days were examined. The results were somewhat variable; 4 of the 6 animals typically showed increased responding in the first component on EXT days, although not on every day. Of the 4, 3 showed a decrease to baseline rates when returned to the RI RI schedule, although 1 of these sometimes didn't return to baseline until the second RI RI day after extinction. With few exceptions, all 6 monkeys exhibited decreased responding in the second component on RI EXT days. Since only three animals were showing behavioral contrast reliably and since the magnitude of the contrast was modest compared with what is normally seen in pigeons and rats, we experimented with a modified schedule in which extinction was introduced randomly in some of the second component bins on the EXT days. This meant that the animal did not know whether or not it would be reinforced during the second component. This procedure produced bursting in the second component in most of the animals the first time or two it was used. However, when we attempted to run a drug experiment, the increased responding was lost in all but one animal on both the no drug and placebo days and we abandoned the random extinction schedule.

Social Behavior and MULT RI RI Schedules. The rank order of the individual monkeys' response frequencies were the same under both the MULT RI RI and MULT RI EXT schedules for total responses and both schedule components. Rank order correlations between operant response frequencies and social behavior scores obtained over 11 days (February 1987) during the diazepam experiment described in the next section showed a strong negative relationship between operant response frequencies and both social rank ($r_s = -.89$) and frequency of submissive behaviors ($r_s = -.94$). Correlations between operant responding and frequencies of responses in the aggressive, other social, and grooming categories of social

behavior were not significant.

Effects of Diazepam on MULT RI RI - MULT RI EXT Performance. Despite the rather uncertain status of the behavioral contrast data obtained initially, an experiment on the effects of diazepam on both the MULT RI RI and MULT RI EXT schedules was conducted using a single dose (0.40 mg/kg) of the drug and incorporating both placebo (vehicle) and no drug days. The data are shown in Table 44, which gives the means and standard errors of the transformed response frequency scores:

Table 44

Diazepam and Performance on Multiple RI 1-min RI 1-min and RI 1-min Extinction 1-min Schedules
(Means +/- SEM of transformed frequency scores [square roots])

A. First Component Responses

Schedule:	Drug Condition: No Injection	Vehicle	.40 mg/kg Diazepam
MULT RI RI	34.38 (7.14)	34.77 (6.82)	30.05 (5.01)
MULT RI EXT	36.17 (8.40)	39.07 (7.74)	36.15 (6.35)

B. Total Responses

MULT RI RI	46.25 (9.60)	47.18 (9.55)	40.03 (6.39)
MULT RI EXT	47.27 (10.84)	50.82 (9.92)	47.28 (8.02)

The two-factor within subjects ANOVAs of both the first component scores and total responses scores yielded a significant main effect of schedule on first component responses ($F_{1,10} = 6.22$, $p < .03$) but not for total responses ($F_{1,10} = 4.54$, $p < .06$). Neither the drug nor the drug by schedule interaction were significant for either measure.

The significant increase in first component responses under the extinction condition indicated that there was a small positive contrast effect in this study. It suggests that additional fine tuning of the schedule parameters might produce a sufficiently reliable contrast effect to make the procedure effective in detecting drug and drug by schedule interaction effects.

Fixed Ratio Schedules:

Fixed ratio procedure. Fixed ratio (FR) schedules whereby the animal is required to make a predetermined number of responses to meet the schedule requirement for receiving each reinforcer were used in conjunction with the study of social behavior in the C-Troop males. An operant panel was attached to one wall of the social observation cage. Initially, banana pellets were delivered on a variable time schedule to encourage the monkeys to increase their social interations in the social test situation. Later, the animals were trained to press a lever to receive pellets during a part of each social test. A typical social test involved starting with a fixed ratio requirement of 10 responses per reinforcer (FR 10) and increasing the requirement to 20 responses per reinforcer (FR 20) halfway through the test. Examples of data obtained with the FR schedules are presented in the section on social behavior.

G. Plasma Hormones - Baseline and Stress:

The project required that physical stressors be imposed at several points in the development of the test battery and that the stress induced by various social manipulations be monitored. Plasma cortisol and plasma prolactin were selected as hormonal indicants of stress. In primates, the primary stress hormone of the adrenal cortex is cortisol. It responds to the imposition of a stressor with a latency of about 15 min, is sensitive to mild stressors, and exhibits little or no habituation to repeated or chronic presentation of the eliciting stimuli. Cortisol has a pronounced circadian rhythm and baseline values tend to be highest in the morning in diurnal primates. Prolactin, from the anterior pituitary, responds to moderate to intense stressors with a latency of about 5 min. It is useful because, unlike cortisol, the magnitude of it response is related to the intensity and/or duration of a stressor. (Kant, Bunnell, Mougey, Pennington & Meyerhoff, 1983). Baseline prolactin levels, which may be elevated at night under some circumstances, tend to be low and stable during daylight hours.

Approximately 850 blood samples were assayed by RIA for both cortisol and prolactin using kits purchased from Cambridge Medical Diagnostics,Inc. Assays were done in the nutrition laboratories of the College of Home Economics at the University of Georgia. Graduate assistants on the project at first assisted the experienced laboratory personnel with the assays and later did them on their own. In the assay for cortisol, recovery ran between 92-96% with intra-assay and inter-assay coefficients of variation of 4% and 7.5% respectively. Prolactin recovery was 98-110% with coefficients of variation of 8.5% and 11.1%.

In obtaining blood samples, the monkeys were restrained during the device mentioned earlier in this report and blood was collected in heparinized tubes from the saphenous veins of the animals' legs. Samples were centrifuged and stored at -18 C until assay. During the first 18 months of the project, samples were taken between 0900-1100 hours. In obtaining baseline data, 2 ml samples were drawn no more often than every second or third day for a period of 10-15 days. During the summer of 1985, a study of diurnal variations in hormone levels was performed with the C-Troop males. Some of the results are described below.

Samples collected from C-, NT-, and I-Troops during 1984 and the winter of 1985 were analyzed in February, 1985. The 230 samples included two social manipulations. In the first of these, Gus, the alpha male in I-Troop was removed for 2 weeks and then returned. The second manipulation involved the introducton of Defeat, a young adult male, into C-Troop.

Baseline prolactin levels were elevated during the first blood draws, but habituated fairly quickly to levels consistent with those we had obtained in some of our previous work in which the samples had been assayed at the Walter Reed Army Institute of Research. Individual monkey's prolactin responses to the social manipulations varied tremendously. Some exhibited large increases, some did not change, and some had slight decreases with respect to baseline values. In rats, prolactin levels have been shown to vary as a function of the intensity and duration of the stressor (Kant, et al, 1983). In our monkeys, we did not find a correlation between either baseline levels of prolactin or changes in prolactin and any of the social variables being measured. Cortisol levels did not habituate very well under baseline conditions and social manipulations often produced readings that were at or near the upper limits of the assay (75ug/100ml). However, our initial concern that there might be a problem with the assay itself, with our handling of the samples, or with the time of day the samples were collected, proved to be unfounded. Reassay of some of the old samples produced interassay coefficients of variation of around 7% and more frequent sampling produced better habitutation.

Because of the high baseline values for cortisol, we were concerned that taking blood during the 0900-1100 hours window was too close to the peak of the diurnal cortisol rhythm and that we would not be able to detect the effects of social manipulations or punishment on hormone levels. We also did not know how frequently we could take blood samples without producing problems in the animals or altering plasma hormone readings. To examine these issues, we did a study with the C-Troop males in which samples were obtained at different times of the day and in which we examined the effects of taking 2 ml samples at intervals of 1, 2, and 3 days on hematocrit values. Table 45 gives the mean values obtained for both cortisol and

prolactin for a series of samples taken at different times.

The plasma cortisol levels were within the normal range seen in other species of macaques, such as rhesus monkeys (Holaday, Meyerhoff and Natelson, 1977) and were 30-50% lower than those we obtained during the first year of the project. As expected, there was a marked diurnal effect such that cortisol values were highest in the morning at the times when we did most of our experimental work. However, the range of response available above these baseline values was deemed sufficient to detect the effects of stressors and other stimuli so that we did not have to worry about a ceiling effect. No circadian rhythm in prolactin was present and none was expected. As noted above, the absolute values for prolactin were comfortably within the range we had obtained from assays done elsewhere on blood samples taken from I-Troop males in 1981.

Table 45

Mean (+/- S.E.M.) Plasma Cortisol and Plasma Prolactin Levels for Six Monkeys Sampled at Four Different Times of Day

Time Window:	0800-0900	1000-1230	1400-1530	2030-2130
Number of Days	4	6	3	2
Cortisol (ug/100 ml)	25.99 (0.81)	26.99 (0.95)	19.42 (1.74)	12.29 (1.21)
Prolactin (ng/ml)	7.83 (1.72)	5.14 (1.05)	6.71 (2.19)	6.88 (1.66)

Hematocrits obtained from the samples in the heparinized tubes averaged about 35-38% and those obtained directly from the vein ran about 43-48%. Hematocrits did not vary appreciably in repeated daily sampling with sample sizes of 2 ml. Thus, we were able to insure that red cell counts were not dropping even with a daily sampling procedure.

Table 46 presents the data obtained from C-Troop following a 20 min exposure to 1.5 ma constant current inescapable footshock delivered on a 1 min variable time schedule and following a 50 min test of social behavior during which time all 6 monkeys were together. There was no overt agonistic behavior during the social test of the group.

Table 46

Mean (+/- S.E.M.) Plama Cortisol and Plasma Prolactin Levels Following Exposure to Shock and Social Group for Six monkeys in C-Troop

	CONTROL	FOOTSHOCK	SOCIAL
Cortisol (ug/100 ml)	21.48 (2.45)	31.83 (0.73)	29.11 (2.32)
Prolactin (ng/ml)	3.71 (0.85)	6.49 (1.84)	3.99 (1.10)

Footshock produced small increases in both cortisol and prolactin. Observation of the animals during the footshock sessions suggested that they were showing considerable behavioral adaptation to the shock. After the first two or three shocks, the monkeys sat immobile and tense for the rest of the session. The marginal increase in prolactin over the control day indicates that the stress produced by footshock was not very great. In the social situation which produced no overt agonistic behavior, there was no rise in prolactin although cortisol levels were up a bit. Since adrenal glucocorticoids tend to respond in all or none fashion to stressors of a wide range of intensities, we do not judge this particular test to have been very stressful.

The cortisol and prolactin values obtained before, during, and after the reintroductions of Cracker and Alabama into I-Troop in the spring of 1985 are presented in Table 47. The reintroduction of Cracker, a low ranking male, produced little overt agonistic behavior in the troop. The reintroduction of Alabama, the second ranked animal, which is described in the social behavior section of this report, resulted in considerable agonistic interaction. For a baseline, 4 samples were taken over eight days prior to Cracker's introduction, 1 on the day of introduction, 1 two days later, 1 on the day of Alabama's introduction four days later, and 3 over the seven days following Alabama's introducton. The values for Cracker and Alabama are not included in the Table until two days after their introductions. Cracker's mean preintroducton cortisol was 14.8 ug/100 ml and on the day of introducton it was 30.9; his prolactin values were 6.1 ng/ml and 21.2. Alabama's baseline cortisol was 34.0 um/100 ml and it rose to 70.6 on the day of introduction. Prolactins were 13.1 ng/ml and 14.8.

Table 47

Mean (+/- S.E.M.) Plasma Cortisol and Plasma Prolactin Levels Following Social Manipulations in I-Troop

	PRE	CRACKER IN	POST	ALABAMA IN	POST
Number of Days	4	1	1	1	3
Cortisol (ug/100ml)	33.00 (2.05)	36.06 (2.98)	33.09 (5.83)	45.73 (6.28)	38.51 (2.92)
Prolactin (ng/ml)	12.95 (2.83)	16.75 (4.67)	24.93 (5.39)	22.37 (6.66)	18.65 (8.23)

Cortisol levels increased following Alabama's introduction and were still slightly elevated eight days later. Cracker's introduction had no effect on mean cortisol on that day. The most interesting effect is the large increase in the standard error that is seen two days after Cracker's introduction and on the day of Alabama's introduction. This means that some animals were responding to the social manipulations with moderate to large increases while others were largely unaffected. This is certainly the case for the prolactin scores where the large increases in standard errors obscure the mean increases following the introductions. There were no significant correlations between individual response frequencies in any social behavioral category and individual values for either hormone. Thus, if social stress is operationally defined by hormone measures, it cannot be assessed by looking at the individual monkey's agonistic response frequencies.

Table 48 presents the hormone data for I-Troop following the 1986 social upheaval described in the section of the report on diazepam and social behavior. Following the fight in early January, 1986, the monkeys were placed in individual cages in the laboratory. A series of 8 blood samples were taken prior to reformation of the group in February, another was obtained immediately following the reintroduction, and 4 more were taken 1, 3, 8, and 15 days post group reformation. Six males are included - samples were not obtainable from Gus at this time.

One way ANOVAs indicated that both cortisol ($F_{4,20}$ = 28.97, $p < .001$) and prolactin ($F_{4,20}$ = 9.63, $p < .01$) were significantly elevated on the day of group reformation. In addition, the posthoc comparisons (Tukey) showed that cortisol on the day after reformation was significantly elevated over the "Group Together" mean. Note that the hormone levels became increasingly variable during social manipulations.

Table 48

Mean (+/- S.E.M.) Plasma Cortisol and Plasma Prolactin Levels During Reformation of I-Troop, Winter, 1986

	ISOLATED	DAY PRIOR TO REFORM	DAY OF REFORM	DAY POST REFORM	GROUP TOGETHER
Number of Days	7	1	1	1	3
Cortisol (ug/100ml)	29.9 (1.4)	29.7 (1.4)	52.7 (3.2)	36.1 (3.3)	25.4 (0.9)
Prolactin (ng/ml)	4.9 (0.8)	4.3 (0.3)	15.5 (3.4)	4.1 (1.1)	5.8 (0.7)

A final sample of the hormone data is shown in Table 49. The top part of the table gives the values of plasma cortisol during the experiment with NT-Troop in which the animal's social behavior was studied under four doses of diazepam. The bottom of the table contains the cortisol level for the group social experiment with C-Troop in which the animals got repeated doses of .80 mg/kg diazepam alternated with placebo. (These experiments were decribed in detail in the section on diazepam and social behavior.)

Table 49

Mean (+/- SEM) Plasma Cortisol Levels with Different Doses of Diazepam

NT-Troop:

	Diazepam Dose mg/kg				
	Placebo	.16	.40	.80	1.60
Cortisol (ug/100 ml)	14.8 (0.5)	12.2 (0.8)	13.9 (1.0)	16.6 (1.4)	13.4 (1.9)

C-Troop:

	Placebo 6 day means	.80mg/kg 4 day means
Cortisol (ug/100 ml)	15.4 (1.1)	12.5 (1.3)

Cortisol levels in NT-Troop became more variable with increasing doses of diazepam, but the differences between the means were not significant. However, alternating doses of .80 mg/kg of the drug with vehicle days produced a small, but

significant decrease in plasma cortisol ($F_{1,5} = 6.66$, $p < .05$).

Overall, the sampling procedures work well and provide reasonable baselines against which to assess the effects of experimental manipulations so long as animals are kept habituated to the procedure. The assays are reliable and valid. Because blood samples are generally drawn in the mornings when the performance testing is done, cortisol baselines tend to be higher. However, the 1986 data shown in some of the preceding tables indicates that baseline levels leave ample room for detecting stress induced increases in both cortisol and prolactin.

Conclusions

Although the amount of time devoted to social observations and analyses was about the same as that spent in performance testing, a larger proportion of this report has been devoted to the evaluation of social observation procedures. The reason for this is that we believe that the major potential contribution of this laboratory and the monkey colony to the objectives of the Medical Research and Development Command is our ability to do sociopharmacological testing. Drugs may affect the behavior of individuals very differently depending upon the both the social status of the individual and the social context in which the effects are observed. The measures of performance on the various laboratory tasks should be of interest primarily because there is always the possibility that differences in social status may affect the way an animal performs on a task. Of course, the effects of drugs on performance can be tested directly, independently of social factors, in our laboratory. However, there are very large number of possible tasks that could be used to assess drug effects. It would seem to be a better strategy to work with a limited number of tasks for which correlations have been established between social variables and performance or which can be used while the animals are in a social situation.

1. Social behavior. We worked with three kinds of social groups during the project: large breeding troops containing all age/sex classes; an all male troop which lived together all the time, and a special troop whose members were housed individually except for short periods each day when they we put together either in twos and threes or as a six member group. Each type of group has both advantages and disadvantages. The large breeding troops provide a more natural and richer social context against which to view drug effects e.g., displaced aggression (scapegoating) and changes in affiliative interactions are more apparent in such a setting. The disadvantage with the larger groups lies in the very large number of possible interanimal relationships we have to keep up

with in order to maintain an accurate record of the social organization of the group. It would be possible to reduce the size of the troops, but doing so reduces the number of animals within a given age/sex class that could be used for evaluating drug effects. The use of a smaller, all male troop made it fairly easy to keep up with interanimal relationships while maintaining a large enough group to allow statistical tests. Agonistic interactions can be studied fairly well, but affiliative behaviors are less frequent and certain kinds of affiliative relationships can't be examined, since age/sex classes other than the adult males are missing. The group in which the animals were together for only limited periods of time allowed us to control the amount of social exposure of the monkeys and to obtain detailed records of dyadic and triadic interactions; however, the overall experience with the social behavior of this group was disappointing. Both agonistic and affiliative behavior frequencies tended to be quite low, except when strange animals were introduced or when competitive food getting and lever pressing was in force. We think that keeping the animals apart so much of the time prevented the formation of strong social relationships and that this was reflected in the reduced sociality and social behavior in this group. We propose to eliminate this type of group from further testing. What we will do is reintegrate them into one or another of the other troops. They can still be used for indoor social observations and activity and operant testing as needed and we expect them to make a stronger contribution to the social data.

Both group scan and focal animal observation procedures are needed to assess social behavior in behavioral pharmacology studies. The focal observations provide for each subject to be observed in the same way and for the same length of time on each occasion. Since focal data are recorded only for the focal animal and the particular animals he is interacting with in the period, data on the social activity among other animals in the group are not available. While this is acknowledged, we were surprised at just how little information about social relationships was obtainable from the focal procedure. We estimate that it takes about five months of focal observations to generate the same amount of information about the breeding troop dominance hierarchies that is obtained with one month of scan observations. Our current procedure of using two observers to obtain simultaneous scan and focal data for all drug studies seems to be the optimal way to gather the social data.

From our pilot experiments with diazepam, it appears that the effects of a given drug on social behavior may differ depending on, among other things, a.) the social context in which the drug is administered i.e., who else is present and the relationships of the subject to these other animals; b) the status of the subject in the dominance hierarchy; c) whether or not the drug is given to all or just some of the animals on a particular day; d) if the latter, who gets the drug on a given

day, e.g., a random selection vs high rank or low rank monkeys; e) whether acute or chronic drug administration is used, and f) the potential for a statistical interaction between dose level and social variables such as rank and level of agonistic activity within the group. Considering these possibilities, we estimate that initial screening of the effects of a new drug on social behavior would take about three months and, should followup experiments be necessary to investigate interactive effects, another three months would be needed. (This assumes that weather conditions would allow a reasonable amount of outdoor testing.) Assuming that criterion, or near criterion, performance on the laboratory tasks would be maintained on a routine basis, the social tests would provide the limiting factor in determining how long it would take to screen a drug.

Removing and replacing selected members of a troop can be used effectively to increase the amount of agonistic behavior in the group without increasing the risk of injury appreciably. Our experience with this procedure in one of the diazepam experiments (I-Troop) indicates that such manipulations need to involve an ABBA series of removals and replacements to make certain that decreases in aggression on the days following an introduction are due to drug effects and not to habituation of the behaviors. Our recent experiences with introducing stranger adult males to an established group indicate that this procedure is more risky, but provides a good way of generating cooperative agonistic behavior. It should probably be used at least once with each drug being screened, but only after basic information as to optimal dose and probable effects across post injection intervals has been obtained.

The introduction of an operant panel to the indoor social testing situation had a salutary effect of increasing social behavior in C-Troop and enabling us to identify both competitive and cooperative interactions among the monkeys. This will be continued in the indoor test situation and the equipment and software for extending these tests to the groups in the outdoor compounds have been assembled and debugged. In fact, we believe that setting up a socalled "closed economy" in one of the outdoor social groups, such that all of the animals would earn their daily food by working on operant schedules, would be both an interesting experiment and, potentially, a valuable addition to the social test battery.

2. Activity Test. We are satisfied with the activity test developed for use with the indoor social observation cage. It has proved very useful for the determination of the initial doses and temporal profiles of drugs to be studied as well as for studies of locomotor activity per se. Since information is often available from other laboratories about drug effects on locomotor activity, the activity test can be used a "ballpark" test to confirm other's results on our animals. It is important, however, that the animals be thoroughly familiar

with the cage before using the test for drug dose determinations and that sufficient testing be done to detect tolerance or sensitization effects. Because we did not pay sufficient attention to these factors in our initial tests, the range of doses we selected for the caffeine studies turned out to be too low.

3. Open Field. Tests of behavior in the open field should be included in the battery. They are sensitive to drug effects, can be correlated with certain social variables, at least in some groups, and they are easy to administer and score reliably. Although the situation can hardly be termed "novel" after the first few exposures, locomotor activity remains fairly high and individual differences in activity scores are readily detected. We found that using a bare open field added little information to that obtained when a new novel object was placed in the field each day and suggest that the novel object be used in all of the routine testing in this situation.

4. Complex Problem Solving. The tests of object quality reversal learning are sensitive to drug effects and are useful in that they enable one to differentiate between habit formation and concept formation. There were significant correlations between social rank, submissive behavior and the number of reinforcers the animals earned. Also, performance varied with atropine as a function of social rank. The use of false reversal trials produced deficits in performance, but the nature of the deficits varied so much from animal to animal that the procedure would not be of much value for screening drugs. Testing on this task using the WGTA is very labor intensive. Although the animals can maintain criterion performance over fairly long periods with a modest amount of refresher training, keeping trained personnel with whom the monkeys are familiar available can be a problem. The task should be automated, or replaced by a similar conceptual task that can be automated.

5. Operant Performance. The DRL task with a modest limited hold requirement as employed in the project should be included in the test battery. It provides for low rates of response and contains a timing component which is useful in assessing drug effects. It's a moderately difficult task requiring response inhibition for successful completion of the schedule requirements; it is easy to detect both improvements and decrements in performance. Since it contains a number of performance elements than can be measured quite readily, it is a good schedule for analytical dissection. Failure of response inhibition, assessed by response bursting, is frequently correlated with aggressive response frequencies in the social situation. We were not asked to evaluate FI schedules in the research protocol but we did so early in the project in order to see if we could replicate other's findings with caffeine. The scalloped response pattern typical of criterion performance

on this schedule is sensitive to drug effects and random omission of reinforcement produces response bursting. In some of our earlier work with rhesus monkeys we found significant relationships between social status and FI preformance (Bunnell, et al 1979a,b). We think that an FI schedule has considerable potential for inclusion in the battery. To do this, we would need more monkeys, or would have to drop one of the other performance tasks from the test battery.

The straight RI schedule, the RI schedule with omission of reinforcement, and the MULT RI RI and MULT RI EXT schedules did not prove to be very useful with positive reinforcement. Performance on the RI schedule did not correlate well with social variables, and random omission of reinforcement produced less response bursting than expected. The RI schedule does produce stable response rates across days and weeks and is susceptible to manipulations which increase or decrease responding. It probably should be a part of the test battery beacuse of its value for assessing drug effects on response suppression to footshock, although behavioral habituaton to footshock (see below) continued to be a problem. The MULT RI RI and MULT RI EXT schedules were evaluated in an attempt to study positive behavioral contrast. We got very little contrast, although some appeared in the diazepam experiment and it may be possible to fine tune the schedule parameters to increase the contrast effect. We got a surprisingly high correlation between between high response rates and both low social rank and submissive response frequencies, but don't know if this will continue to hold up across repeated testing. Since the RI schedule is useful in the punishment paradigm and it is easy to shift the animals to the MULT RI schedule, additional evaluation of the latter seems warranted. The alternative would be to look at a different multiple schedule, such a MULT RI DRL schedule.

Two attempts were made to investigate aversive schedules using the C-Troop males. We ran into two problems trying to investigate free operant (Sidman) avoidance. The monkeys showed rapid behavioral habituation to footshock and we had difficulty getting them to attend to the cue stimuli we were using to try to shape the lever pressing reponse. We believe that the problems can be overcome, but that the most efficient way to test performance on either Sidman avoidance or a conditioned suppression task would involve restraining the monkeys in primate chairs for extended periods of time. Chairing the animals is inimical to the investigations of social behavior and the procedure is getting harder and harder to justify to institutional animal care and use committees. (In fact, we have had to get permission to chair wounded animals undergoing treatment by our consulting veterinarian.) Fortunately, the random punishment schedule used with the FI schedule produces good suppression and is sensitive to the anxiolytic effects of drugs.

6. Stress Hormones. Both plasma cortisol and plasma prolactin provide good indices of social and behavioral stress. As expected, cortisol is a good index of general arousal and/or mild stress, such as is seen in the open field tests. Prolactin responds in a graded fashion to more intense stressors such as agonistic encounters, footshock, and the like. Prolactin is also more variable within subjects and will habituate more quickly across days. Sampling time, in terms of the delay between the time the animal is removed from the test situation and the sample is obtained need to be as constant as possible and this sometimes presents a problem when we do post social test draws on several animals in a troop since they cannot all be sampled at the same time. Nevertheless, the data are generally very good. We are presently evaluating an assay for ACTH in conjunction with another project and this could be added to the test battery if it proves useful. Of more interest for the future is a recent report that social (interview) stress in humans increases plasma levels of the second messenger cyclic AMP (Meyerhoff, Oleshansky and Mougey, 1988). The authors suggest that the increase reflects activation of adrenergic receptors. If this proves to be the case, then assays for plasma cyclic AMP might be a useful addition to the test battery. We have been doing some work on plasma cAMP in rodents and there is evidence suggesting that it increases in a graded fashion to increasing levels of a stressor.

7. Potential Additional Tests. In examining the data from the initial tests evaluated, it is apparent that some tests that would tap additional dimensions of performance should be added. A test of vigilance using either auditory or visual stimuli should be relatively simple to set up and evaluate and might be related to the animal's readiness and ability to respond to other animals in a social situation. In another area, one of our graduate assistants has evaluated visual preferences in baboons using social stimuli (Kyes and Candland, 1984). Mr. Kyes has set up a similar apparatus and used some of our young females as pilot subjects. The object has been to see if the animal prefers members of its own troop to others, high to low ranking animals, relatives to nonrelatives, same age/sex to different age/sex class, etc. Since the diazepam testing seemed to show that animals responded to each other differently when given the drug, a laboratory task of this sort could provide useful data about the ways drugs affect responses to social cues and signals. Finally, as noted above, we think that the major thrust of any additional work in this area should be directed at performance testing in social group situations. Additional work on developing cooperative tests would be an important part of such a project.

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APPENDIX A

Social Behavior in I-Troop: The following tables are the matrices derived from the analyses of 11 days of 40 min group scan social data recorded from I-Troop during the period 1 July - 16 July 1985. I-Troop was intact during this time - all 8 adult male monkeys were present during all observation periods. The matrices labeled SUBMISSIVE, AGGRESSIVE, NONAGONISTIC SOCIAL, and SEXUAL contain the behaviors listed in these categories in Table 2 of the main text. The GROOMS matrix is for social grooming (allogrooming) and contains this behavior only. GROOMS is contained within the NONAGONISTIC matrix as well. In each matrix, the frequency with which each monkey directs a given class of behavior toward every other animal in the troop is read across the horizontal rows. The frequency with which each monkey receives each class of behavior is read down the vertical columns. Row, column, and matrix totals are at the right margin and the bottom of each matrix. The SUBMISSIVE matrix establishes the social rank hierarchy in terms of who submits to whom. The other matrices are constructed using this same order. Notice that the SUBMISSIVE matrix shows that 27 of the 28 possible dominance/submission relationships have been identified during the 11 observation periods. Only the relationship between Yuk and Quotation was not directly observed.

SUBMISSIVE:

	GUS	ALABA	SPIRO	YAMAM	CRACK	YUK	QUOTA	EQUAL	TOTAL
GUS		0	0	0	0	0	0	0	0
ALABAMA	2		0	0	0	0	0	0	2
SPIRO	1	1		0	0	0	0	0	2
YAMAMOTO	5	4	5		0	0	0	0	14
CRACKER	2	4	7	1		0	0	0	14
YUK	1	2	2	6	1		0	0	12
QUOTATION	2	4	7	5	3	0		0	21
EQUAL	5	6	9	6	7	7	2		42
TOTAL	18	21	30	18	11	7	2	0	107

APPENDIX A

AGGRESSIVE:

	GUS	ALABA	SPIRO	YAMAM	CRACK	YUK	QUOTA	EQUAL	TOTAL
GUS		0	0	5	4	2	2	0	13
ALABAMA	0		0	2	0	0	4	0	6
SPIRO	0	0		5	4	0	3	0	12
YAMAMOTO	0	0	0		0	5	0	0	5
CRACKER	0	0	0	0		4	4	6	14
YUK	0	0	0	0	0		1	13	14
QUOTATION	0	0	0	0	0	0		1	1
EQUAL	0	0	0	0	0	0	0		0
TOTAL	0	0	0	12	8	11	14	20	65

NONAGONISTIC SOCIAL:

	GUS	ALABA	SPIRO	YAMAM	CRACK	YUK	QUOTA	EQUAL	TOTAL
GUS		37	1	3	2	1	0	0	44
ALABAMA	11		6	4	2	4	6	5	38
SPIRO	0	1		55	6	39	37	35	173
YAMAMOTO	0	0	24		7	61	7	29	128
CRACKER	6	5	3	0		4	35	78	131
YUK	0	0	2	23	0		6	6	37
QUOTATION	0	0	1	1	8	0		1	11
EQUAL	0	1	18	4	37	25	4		89
TOTAL	17	44	55	90	62	134	95	154	651

APPENDIX A (Continued)

GROOMS:

	GUS	ALABA	SPIRO	YAMAM	CRACK	YUK	QUOTA	EQUAL	TOTAL
GUS		16	0	1	0	0	0	0	17
ALABAMA	3		3	1	0	1	2	3	13
SPIRO	0	0		21	0	19	16	14	70
YAMAMOTO	0	0	16		1	23	0	12	52
CRACKER	2	1	2	0		1	15	21	42
YUK	0	0	0	15	0		2	10	17
QUOTATION	0	0	0	1	2	0		0	3
EQUAL	0	0	11	1	29	13	1		55
TOTAL	5	17	32	40	32	57	36	50	269

SEXUAL:

	GUS	ALABA	SPIRO	YAMAM	CRACK	YUK	QUOTA	EQUAL	TOTOL
GUS		0	0	0	0	0	0	0	0
ALABAMA	0		0	0	0	0	2	5	7
SPIRO	0	0		0	0	1	0	1	2
YAMAMOTO	0	0	0		0	0	0	0	0
CRACKER	0	0	0	0		0	0	0	0
YUK	0	0	0	0	0		0	0	0
QUOTATION	0	0	0	0	1	0		0	0
EQUAL	0	1	2	0	0	0	0		3
TOTAL	0	1	2	0	1	1	2	6	13

APPENDIX B

Effects of 15 min vs 30 min Delays Between Atropine Sulphate Injection and Start of Testing on DRL Performance

	DOSE mg/kg							
	.08			.20				SALINE *
Min Delay:	15	30(1)	30(2)	15(1)	15(2)	30(1)	30(2)	(+/- SEM)
Animal								
a. Efficiency Index:								
Barker	.26	.38	.16	.16	.30	.11	.08	.58 +/-.01
Eju	.80	NR	.39	.30	.67	.06	NR	.59 .03
Hobbit	.28	.48	.27	.17	.23	.28	.33	.45 .03
Tag	.06	.16	.15	.13	.13	.12	.12	.17 .01
Allen	.33	.39	.56	.41	.38	.37	.33	.41 .03
Kukla	.07	.25	.32	.15	.07	.19	.13	.32 .06
Weed	.22	.40	.30	.29	.30	.10	.31	.43 .04
b. Number of Reinforcements out of 40:								
Barker	13	40	17	3	5	13	3	40 +/- -
Eju	4	NR	27	3	2	2	1	40 -
Hobbit	40	40	40	36	40	40	40	40 -
Tag	10	40	40	19	17	21	40	40 -
Allen	34	40	40	32	29	38	3	37 2.4
Kukla	9	40	23	8	9	16	2	39.5 0.3
Weed	39	28	30	23	25	11	14	40 -
c. Limited Hold Exceeded:								
Barker	152	64	124	142	121	136	139	8.9+/-3.2
Eju	166	NR	113	147	127	157	152	22.3 2.8
Hobbit	69	27	31	91	63	55	39	18.8 4.7
Tag	152	7	33	117	99	131	19	29.1 13.1
Allen	116	25	67	107	79	108	149	42.4 16.6
Kukla	147	60	118	136	99	137	141	47.7 4.7
Weed	82	98	78	100	92	107	109	18.5 8.6
d. Bursting (# 1st bin responses):								
Barker	12	27	7	15	5	70	18	10.7+/-1.3
Eju	0	NR	9	2	1	21	0	9.4 3.8
Hobbit	64	28	62	9	89	62	17	26.7 2.8
Tag	124	157	200	143	87	115	246	186.1 13.2
Allen	9	14	11	6	7	15	2	21.2 2.2
Kukla	81	58	21	26	88	41	5	44.7 10.5
Weed	71	17	38	23	34	40	13	28.7 6.3

APPENDIX C

Personnel and Other Matters

1. The following people were employed on the project:

Name	Position	% Effort	Dates Employed
B. N. Bunnell	PI	25%	9/83-12/86
W. B. Iturrian	Co-PI	10%	9/83-12/86
S. C. Baker	Grad Asst.	33%	7/85-12/86
W. E. Hills	Grad Asst.	33%	10/83-10/85 & 7/86- 8/86
R. C. Kyes	Grad Asst.	33%	9/85- 9/86
T. E. Orr	Grad Asst.	33%	6/84- 9/86
D. E. Reddick	Grad Asst.	33%	10/83- 9/85
S. H. Snodgrass	Grad Asst.	33%	7/86- 9/86
R. W. Shumaker	Student Asst.	25%	11/83- 6/84
I. Rosenberg	Student Asst.	25%	6/86- 8/86
T. L. Peacock	Lab Technician	100%	2/84- 5/86
J. R. Harris	Caretaker	100%	11/83- 7/85
E. H. Wooley	Caretaker	100%	8/85- 9/85
D. E. Reddick	Caretaker	100%	9/85-11/85
M. D. Beatey	Caretaker	100%	11/85- 6/86
	(Beatey on U.GA payroll		7/86-12/86)

2. No graduate assistant did his/her dissertation as a part of this project. However, Baker, Hills, Orr and Snodgrass have since completed the PhD and the support they received while working on the contract made a significant contribution to their success in graduate school.

3. To date there have been no publications resulting from work done on this contract. As such appear, we will submit four copies of each to the Command.

APPENDIX D

Executive Summary

This report summarizes work accomplished during a three year project, the goal of which was to develop a battery of tests of social behavior and performance for nonhuman primates that would be sensitive to the effects of CW-related chemicals considered for use as antidotes or therapeutics against CW agents. Different procedures for assessing social behavior are described and evaluated, as are a number of tests for emotional reactivity, complex problem solving, and performance on appetitive and aversive operant schedules. Data are presented on changes in plasma hormone levels in response to manipulations of social and performance variables designed to induce stress in the subjects. The suitability of the test battery for use in studying CW-related chemicals was evaluated using an antidote (atropine sulphate), a therapeutic (diazepam), and control drugs (caffeine and atropine methyl nitrate).

The majority of the behavioral testing was done with 28 adult and subadult male cynomolgous macaque monkeys (*Macaca fascicularis*) who comprised the adult male social hierarchies of four captive groups of animals. Two of the groups were breeding troops containing females, juveniles and infants in addition to the adult males. The other two groups were all male troops. Several procedures for gathering and analyzing social behavior data were evaluated in terms of their utility for drug studies as were different methods of manipulating the amount and kind of social behavior exhibited by the monkeys. These included methods of inducing cooperative agonistic and cooperative affiliative behavior in the groups.

A test of general locomotor activity and a procedure for selecting the inital doses of drugs to be used with behavioral protocols was developed and evaluated. An open field test for studying emergence and response to novel stimuli was evaluated and judged worth including in the test battery.

A test utilizing object quality and reversal learning sets to study complex problem solving was sensitive to drug effects and was useful in differentiating between habit formation and concept formation. Some aspects of performance were correlated with social variables and there was an interaction between social rank and atropine effects on peformance. Because the test as presently adminstered is very labor intensive, it is recommended that the task be automated if it is to be kept in the battery.

Of the several operant reinforcement schedules examined, a differential reinforcement of low rate of response (DRL) schedule which incorporated a limited hold requirement was selected for inclusion in the battery because of its sensitivity to drugs and correlation with social variables. Similar characteristics led to a suggestion for including a fixed interval (FI) schedule as well. Results with random interval (RI) schedules, with and without omission of reinforcement to produce response bursting, were somewhat disappointing since little bursting occurred. However, the steady response rates produced by the schedule were sensitive to drug effects and the schedule is particularly useful for studying response suppression to aversive stimuli (e.g., footshock). Multiple random interval (MULT RI RI) and random interval extinction (MULT RI EXT) schedules failed to produce the hoped for positive behavioral contrast; however, one study with diazepam indicated that some contrast was occurring and it was suggested that additional fine tuning and evaluation of multiple schedules would be worthwhile. There were some high correlations between social variables and the MULT RI RI schedule that should be examined further. Attempts to train the monkeys on free operant avoidance and conditioned suppression tasks were not successful because they showed rapid behavioral habituation to footshock in the test chambers and because it was difficult to get them to attend to cue stimuli. It appeared that success with these tasks would require that the monkeys be confined to primate chairs for training and testing, a situation inimical to the social testing protocols. Fortunately, the response suppression procedure described above worked well. Performance on fixed ratio (FR) schedules in a social group was evaluated with one troop of monkeys and proved useful for generating increased social behavior and enabling the identification of both competitive and cooperative interactions amomg the monkeys.

A large number of blood samples were obtained from the monkeys throughout the project. Assays for plasma prolactin and plasma prolactin showed that these hormones were, as expected, good inuices of social and behavioral stress. It was suggested that measures of levels of ACTH and plasma cyclic AMP be added to the battery. The latter might be particularly useful in assessing activation of adrenergic receptors.

The possibility of adding additional tests to the battery, including a vigilance task, a preference test for social stimuli, and developing additional ways of studying performance in social situations, was proposed.

The primary contribution that the laboratory and the monkey colony can make is in the area of sociopharmacological testing. An animal's social status may affect the way it performs on a task and drugs may affect the behavior of individuals very differently depending on the social status of the individual and the social context in which the behavior is observed. The potential for such interactions is important for determining drug effects on behavior and performance. The best approach will be to use tasks in which drug effects on performance can be studied directly in social group situations.

As long as the resources are available to maintain criterion, or near criterion, performance on the laboratory tasks, the limiting factor in determining how fast a new drug can be screened will be the various social behavior tests and manipulations. We estimate that performance testing in the laboratory can be completed in about three months. Depending upon the nature of the drug/social variable/performance interactions, social testing will take from three to six months.

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